Application of Compost from Oil Palm Empty Fruit Bunch and Palm Oil Mill Effluent Anaerobic Sludge in Oil Palm Plantation as Nutrients Recycling

APPLICATION OF COMPOST FROM OIL PALM EMPTY FRUIT BUNCH AND PALM OIL MILL EFFLUENT ANAEROBIC SLUDGE IN OIL PALM PLANTATION AS NUTRIENTS RECYCLING

SITI SULIZA BINTI SALAMAT

DEPARTMENT OF BIOLOGICAL FUNCTIONS AND ENGINEERING
GRADUATE SCHOOL OF LIFE SCIENCE AND SYSTEMS ENGINEERING
KYUSHU INSTITUTE OF TECHNOLOGY
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By

SITI SULIZA BINTI SALAMAT

Department of Biological Functions and Engineering
Graduate School of Life Science and Systems Engineering
Kyushu Institute of Technology
2021

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LIST OF ABBREVIATIONS

AAS	Atomic absorption spectroscopy
Al	Aluminum
B	Boron
Bp	Base pair
BLAST	Basic logical alignment search tool
Ci	Calcium
Cu	Cuprum
cm	centimeter
cm^2	square centimeter
cm^3	cubic centimetre
CEC	Cation exchange capacity
CMC	Carboxymethylcellulose
COD	Chemical oxygen demand
CPO	Crude palm oil
DGGE	Denaturing gel gradient electrophoresis
DAP	Diamonium phosphate
DNA	Deoxyribonucleic acid E.coli Escherichia coli
dNTP	deoxynucleotide triphosphate
EFB	Empty fruit bunch
Fe	Ferum
FASSB	Felda Agricultural Services Sdn Bhd
FELDA	Federal land development authority
FFB	Fresh fruit bunch
g	gram
HGFB	High grade fertilizer borate
K	Potassium
kg	kilogram
KCI	Muriate potash
L	liter
m	meter
mL	milliliter
mg	milligram
Mn	Manganese
Mg	Magnesium
MEGYP	Maximum exploitation of genetic yield potentials
MOP	potassium chloride
MPOB	Malaysian Palm Oil Board
MPOC	Malaysian Palm Oil Congress
N	Nitrogen
NGS	Next –generation sequencing
NCBI	National Center for Biotechnology Information
OPEFB	Oil palm empty fruit bunch
OTU	Operational Taxonomical unit
OPT	Oil palm trunks
OPF	Oil palm fronds

P	Phosphorus
PCoA	Principal coordinates analysis
PCR	Polymerase chain reaction
POME	Palm oil mill effluent
Qiime	Quantitative insight into microbial ecology
RCBD	Randomized complete block design
RM	Ringgit Malaysia
RDP	Ribosomal database project
RP	Rock phosphate
RSPO	Rountable on Sustainable of palm oil
RT-PCR	Real-time polymerase chain reaction
rRNA	Ribosomal ribonucleic
S	Sulfur
Si	Silica
SIRIM	Standard & industrial research institute of Malaysia
SMS	Synthetic magnesium sulphate
SMP	Soluble microbial products
TSP	triple superphosphate
Zn	Zink
%	Percentage

PUBLICATIONS AND CONFERENCE ATTENDED

1. Siti Suliza Salamat, Mohd Ali Hassan, Yoshihito Shirai, Ahmad Husni Mohd. Hanif, Mohd Shahkhirat Norizan, Mohd Huzairi Mohd Zainudin, Nurul Asyifah Mustapha, Mohd Noor Mat Isa, and Mohd Faizal Abu Bakar (2021). Effect of Inorganic Fertilizer Application on Soil Microbial Diversity in an Oil Palm Plantation. *BioResource*, 16, 2279-2303 (IF: 1.409).

2. Siti Suliza Salamat, Mohd Ali Hassan, Yoshihito Shirai, Ahmad Husni Mohd. Hanif, Mohd Shahkhirat Norizan, and Izwanizam Arifin (2019). Application of Compost in Mixed Media Improved Oil Palm Nursery`s Secondary root structure thereby Reducing Root Structure Thereby Reducing the Fertilizer Requirement for Growth. *AsPac J. Mol Biology Biotechnol*, 27, 39-49 (Scopus Index Journal).

3. [Poster] Siti Suliza Salamat, Mohd Ali Hassan, Yoshihito Shirai, Ahmad Husni Mohd Hanif, Izwanizam Arifin, Mohamad Shahkhirat Norizand (2014). The Effects of 25 Years of Inorganic Fertilizer Regimes on Soil Properties Composition on Oil Palm. Symposium on Applied Engineering and Sciences (SAES 2014), UNIVERSITI PUTRA MALAYSIA.

4. [Oral presenter] Siti Suliza Salamat, Mohd Ali Hassan, Yoshihito Shirai, Ahmad Husni Mohd Hanif, Izwanizam Arifin, Mohamad Shahkhirat Norizand (2017). Application of Compost Enhanced the Secondary Root Structure Which Reduced the Fertilizer Requirement in Oil Palm Main Nursery. Applied Engineering and Sciences (SAES 2017), UNIVERSITI PUTRA MALAYSIA.

ABSTRACT

Malaysia is the world's second largest producer of palm oil. For every tone of crude palm oil, more than 3 tons of effluent is produced. The general thinking in the palm oil industry is that in order to increase the oil yield, more chemical fertilizers should be applied. This has caused increased consumption and excessive chemical fertilizer application at the plantations that eventually led to environmental pollution. At the same time, empty fruit bunch (EFB) and oil palm mill effluent (POME) anaerobic sludge are potential sources of raw material for the production of organic fertilizer. Although oil palm empty fruit bunches (EFB) as organic compost supplemented with inorganic fertilizer has been practiced in oil palm plantations, there is little evidence to support its effectiveness. In the absence of technical information, estates are applying large amounts of N, P and K fertilizers with the EFB due to the need to maintain high oil yield. At the same time fertilizer wastage occurs when excess fertilizer is lost by run-offs when it rains. Raw POME has been used as supplementary fertilizer in oil palm plantation as land application since POME contains some essential elements such as Ca, Mg, K, P and N and some micronutrients. POME contains water that enables it to reduce water deficit during the dry season. This study was initiated to study the effects of oil palm EFB together with POME anaerobic sludge on oil palm growth and yield and the soil chemical properties. The goal is to develop EFB and POME anaerobic sludge as organic fertilizer to improve crop yields, reduced fertilizer costs, increased soil fertility and reduced environmental pollution. The project is divided into three parts.

Firstly, the soil characteristics in the oil palm plantation after 25 years of cultivation was compared to secondary forest soil as control. The results showed that the soil characteristics, especially the pH, were not significantly different. Regarding the bacterial community, the kingdom Achaea was only present at secondary forest. In the secondary forest soil, the phyla *Firmicute*s and *Bacteroidetes* were higher compared to *Proteobacteria*. In the oil palm plantation soil after 25 years of inorganic fertilizer application, the phyla *Proteobacteria* and *Actinobacteria* were high, whereas *Firmicute*s and *Bacteroidetes* were low.

Secondly, different percentages of chemical fertilizer and compost fertilizer were then tested on the oil palm plantation over a period of 5 years. The results showed that application mixed inorganic fertilizer, even with 100% organic fertilizer, did not affect plant growth, soil and oil yield of oil palm. Achaea which is normally found at secondary forest appeared after four years application in treatment with 50% inorganic fertilizer: 50% organic fertilizer, 25 % inorganic fertilizer: 75 % organic fertilizer and 100 % organic fertilizer. The organic fertilizer increased the abundance of *Firmicute*s and *Bacteroidetes* as good bacterial indicators of soil. On the economic analysis, 50% inorganic fertilizer: 50 % organic fertilizer is compatible with 100 % inorganic fertilizer. Inorganic fertilizer from biomass of oil palm can save almost 50% cost of imported inorganic fertilizer.

Thirdly, in the oil palm main nursery study, the results showed that 50 % soil: 50 % compost with 100 % inorganic fertilizer can be adopted as commercial

practice by the palm oil industry. Mixed media with 50 % soil: 50 % compost can maintain the nutrient composition in the soil and trigger plant growth comparable to 100 % soil with 100 % inorganic fertilizer. Treatment 2, mix media 50 % soil: 50 % compost with 100 % inorganic fertilizer showed microbial diversity same patent with mix media 50 % soil: 50 % soil with 75 %, 50 % and 25 % inorganic fertilizer. It is means suitable media and application inorganic fertilizer encouraging accumulation of microbial activity. The cost per polybag with 50 % soil: 50 % compost with 100 % inorganic fertilizer is RM 5.20, compared to 100 % soil with 100 % inorganic fertilizer at RM6.04; i.e. with a reduction RM0.84 per polybag.

Overall, the results in this study showed that publication of organic fertilizer did not decrease the oil extraction rate of oil palm fruit. The data obtained suggests that 50% application of organic fertilizer from EFB and POME anaerobic sludge oil palm plantation not only reduced the cost of inorganic fertilizer, but can also resolve environmental problem from the waste of palm oil mill. Furthermore, the results of the study showed the impact of organic fertilizer application can increase the fertility or soil by facilitating the growth

CHAPTER 1

INTRODUCTION

1.1 Overview of research

The oil palm (*Elaeis guineensis* Jacquin) is economically important for Malaysia for its oil, whereby Malaysia has become the second biggest exporter in the world after Indonesia. The climate, soil and agro-ecological zone in Malaysia are suitable for oil palm. Malaysia has a total land area of 329,733 km^2 which is divided into two geographical regions, Peninsular with land area of 131,573 km^2 and East Malaysia which comprises Sabah and Sarawak with land of 73,711 km^2 and 124,449 km^2. Malaysia has warm humid tropical climate throughout the year. Humidity is about 85% with temperature range from 21-32°C, annual rainfall 2,450 mm and above and year round day length of 12.5 hours (Lim *et al*. 2011). The soil in Malaysia can be divided broadly into 2 main groups, sedentary soil from rock type and soil of the coastal alluvial plains.

Climate requirement for oil palm is high rainfall between 200 and 300 cm/year, 15cm of rainfall is requirement for each month without distinct drought season or months with less than 10cm rain (Goh 2004). Optimal temperature range between 22°C and 33°C with the lowest temperature supporting the plant close to 20°C (Goh 2004). The daily requirement of sunlight is between 5 and 7 daylight hours and at least 2000 hours of sunshine annually (Lim *et al.* 2011). Relative humidity should between 75 and 100 percent (Lim *et al.* 2011). Most soil are suitable and the crop does not demand high fertility soil, except the soil should not be heavy with large amount of clay due to water logging during the monsoon season (Lim *et al.* 2011). Suitable soil texture is sandy

loam of more than 75cm depth. Lateritic, sandy or peat soils are problematic soils that need proper manuring and maintenance for optimum palm growth (Tan et al. 2014). Ideally, oil palm should be grown in flat areas. For inland soils, planting is done in a triangular form, with a distance of 8.8 m, giving 148 palms/ha, coastal alluvial soil is 136 palms/ha and peat soils 160 palms/ha.

Consequently, fertilizers are essential for economic production as attested by field experiments and growth in fertilizer usage in the oil palm sector. For good yields to be sustained, fertilizer inputs are necessary and typically constitute 40-50 % of total field upkeep cost. With palm oil projected to grow to 35 million tons by 2020, the expansion in fertilizer requirements is assured and this makes pleasant news to people in the trade. Most of fertilizers used in Malaysia are imported. Urea was imported from Indonesia, Vietnam, China, Saudi Arabia, Thailand, Australia, India, Japan and Philippines. Ammonium sulphate imported from Japan, China, Russia, Korea, and Taiwan. Rock phosphate imported from Egypt, Tunisia, Algeria, Christmas Island and Australia.

At the same time, both the expected increase in palm oil production and concomitant fertilizer usage have to take full cognizance of worldwide environmental concerns on two major counts. The first focuses on huge quantities of biomass by-products are also generated annually and renewable source is not fully wisely utilized, while the second concerns pollution of water and the air by agro-chemicals, including fertilizers. In the 2012, from the 82.39 million tons of total fresh fruit bunch processed, the biomass produced are as follows: shell 4.94 million tons, fiber 9.87 million tons, empty fruit bunch 18.13 million tons and palm oil mill effluent (POME) 49.43 tons.

1.2 Problem statement

Every year, Malaysia's oil palm industry has to import a large amount of chemical fertilizers to meet the needs for the growth of oil palm trees. Statistics recorded by Food and Agriculture Organization of the United Nations (2016) states that in 2001, Malaysia imported RM 1144 million worth of chemical fertilizers. The reasons included by Food and Agriculture Organization of the United Nations (FAO) is Malaysia lacks the raw materials of P and K. Although Malaysia exports 1.75 million tones urea, Malaysia still imported 386,571 million tons per year urea for local use from Russian Federation Company. In addition to that, it is proven in many studies that prolonged use of chemical fertilizer is detrimental to the soil fertility (Ge *et al*. 2018). In order to mitigate the problem, a partial substitution of the chemical fertilizer with the use of compost was proposed. Furthermore, the use of EFB and POME sludge to the composting process also solved some waste management problems faced by the industry. Therefore, this research aims to determine the effect of compost as a partial substitute for chemical fertilizers to the oil palm at nursery and growth stage and to determine its effect on the soil characteristics and microbial profile.

1.3 Research objectives

The objectives of this research are:

1. To conduct baseline study, characterize macronutrient, micronutrient and microbial diversity profile to soil planted with oil palm plantation.
2. To evaluate the effect of organic and inorganic fertilizer on changes of oil yield, the physical characteristics of oil palm, soil microbial diversity profile, oil extraction and economical statistic.
3. To determine the effect of compost as media to reduce inorganic fertilizer used at oil palm nursery stage.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

In this chapter, some information on oil palm plantation until production are discussed. A review on oil palm plantation recovery on oil palm plantation is made on recent issues on industrial oil palm, waste of oil palm (empty fruit bunch and POME sludge) use as inorganic fertilizer and on microbial and economic study as well as oil extraction. The soil grown with oil palm plantation in Malaysia mainly belongs to order Ultisols and Oxisols which are predominantly low in fertility and are acidic (Shamshuddin *et al.* 2012). Fertilizer cost constitutes 30-50% of the total production costs of fresh fruit bunches (FFB) of oil palm in Malaysia (Lee *et al.* 2003). Therefore, reducing the fertilizer cost by the use of appropriate fertilizer is welcomed. Increasing FFB yields will lead to enormous economic benefits. Almost 90% of farming system in Malaysia used mineral or inorganic fertilizers mainly in conventional forms (FOA, 2004). Oil palm production needs large quantities of fertilizers to get good yield (Comte *et al.* 2012). Fertilizer management on undulating and hilly soils for oil palm plantation is very challenging because of the need to maintain fertility, minimize soil erosion and nutrient loss on oil palm plantation. Another important issue in Malaysia is about frequency of application of large amounts of chemical fertilizer, due to high rainfall intensity which increased the risk of nutrient loss. The loss of nutrients through leaching and runoff reduced both crop productivity and economic gains. There is a need to develop alternatives from the fertilizer industry in Malaysia to cater for

fertilization of high value crops such as oil palm in order to make it more economically viable and ecologically compatible.

2.1.1 Overview of oil palm plantation in Malaysia

The African oil palm is native to tropical Africa, from western Sierra Leone to eastern Democratic Republic of the Congo. It was domesticated in its native range, probably in Nigeria, and moved by humans throughout tropical Africa who practiced agricultural shifts at least 5000 years ago. The palm was discovered by European explorers in the late 1400s and spread throughout the world during the slave trade period. The slave trade ended in the early 1800s but British continued to exchange gold, cotton and palm oil with West Africans. The oil palm was introduced to the Americans hundreds of years ago, where it became naturalized and synonymous with slave plantations, but did not become and industry of its own until the 1960s. Oil palm is a family of monocot plant under the genus of Elaeis, a subfamily of Cocoidea. Since the plant is monocot, the leaves have parallel veins and have C4 type of photosynthesis. C4 photosynthetic pathway involves both mesophyll cell and its bundle sheath rather than C3 photosynthetic pathway that uses only its mesophyll cell by the enzyme Rubisco for glucose production. The classification of oil palm for the subfamily of Cocoidea is due to fact that the fruit is unarmed except some short spines at the base of the leaf and within the branch. Furthermore, oil palm is mainly classified according to the fruit internal structure (Hartley, 1988). The first oil palm plantations were established in in Sumatra Indonesia in 1911, followed by Malaysia in 1917. Around this time, oil palm plantations were developed in tropical America and West Africa, and in 2003 the production of palm oil was equivalent to that of soybean, the number one oil crop for many years.

The oil palm (*Elaeis guineesis*) was first introduced as an ornamental plant in Malaysia in 1870. Planting area had risen at a rapid rate since 1960. The oil palm has an economic value for its oil and has become one of the world's major oil crops. Global vegetable oils by Statista 2021 is palm oil 75.45 million metric tons, followed by sunflower seed oil19.02 million metric tons, palm kernel oil 8.51 million metric tons, peanut oil 4.89 million metric tons, cotton seed oil 4.89 million metric tons, coconut oil 3.67 in million metric tons, olive oil 3.1 million metric tons, and rapeseed oil 27.64 million metric tons.

2.2 Performance of oil palm plantation in Malaysia

Malaysia's oil palm sector began in a modest manner about 100 years ago. It was first introduced as a commercial plant at the Tennamaram Estate in Selangor to Malaya (now Malaysia) in 1917, which effectively laid the foundation for the growth of the oil palm industry in Malaysia. Figure 2.1 shows the development of the palm oil production increased considerably from below 100 000 t in 1960 to around 17.32 million tons in 2016 (MPOB, 2017). In 2016, Peninsular Malaysia accounted for about 47 percent of the planted region, Sabah for 27 % and Sarawak for 26%. In Table 2.1, according to category, oil palm planted area by private estate accounted for the biggest planted area of 3.51 million hectares in 2016, covering 61.2%, followed by autonomous smallholders of 0.93 million hectares (16.3%), Felda of 0.71 million hectare (12.3%), government schemes of 0.34 million hectares (6.0%), Felcra of 0.17 million hectares (3.0%), and RISDA of 0.07 million hectares (1.2%).

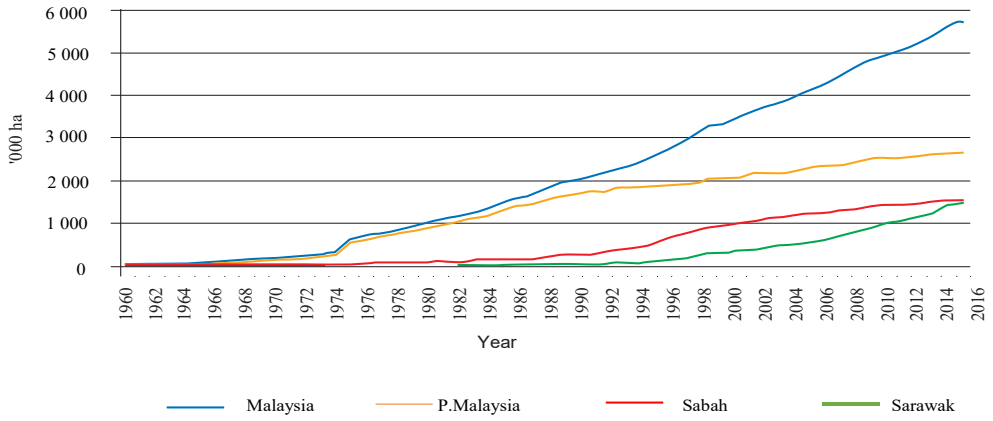


Figure 2.1 Oil palm planted in Malaysia from 1960 until 2016

Source: (MPOB 2017)

Table 2.1 Oil palm planted area according to category in Malaysia in 2016 (ha)

Private estates	3 508 554	61.2
Government schemes		
Felda	706 588	12.3
Felcra	173 032	3
RISDA	71 549	1.2
State schemes	344 314	6
Independent smallholders	933 948	16.3
Malaysia (Total Area)	5 737 985	100

Note: Felda - Federal Land Development Authority.
Felcra - Federal Land Consolidation and Rehabilitation Authority.
RISDA - Rubber Industry Smallholders Development Authority.

Source: (MPOB 2017)

2.2.1 Production of crude palm oil (CPO)

After three years of planting, the oil palm begins producing oil, and reaches its peak output at 12 to 15 years of planting after which the yield begins to decline. Although the suggested age of replanting oil palm is at 25 years, the choice to replant is often based on variable factors, including palm productivity, problem of plant height, manufacturing expenses and cost. The quality of FFB output will be affected by management and agriculture practices. Weather and good farming practices will lead to better FFB yield and greater manufacturing of CPO (Rahman et al., 2013). Figure 2.2 shows the increase in planting area to 5.74 million hectares in 2016 and CPO production increased to 17.32 million tons.

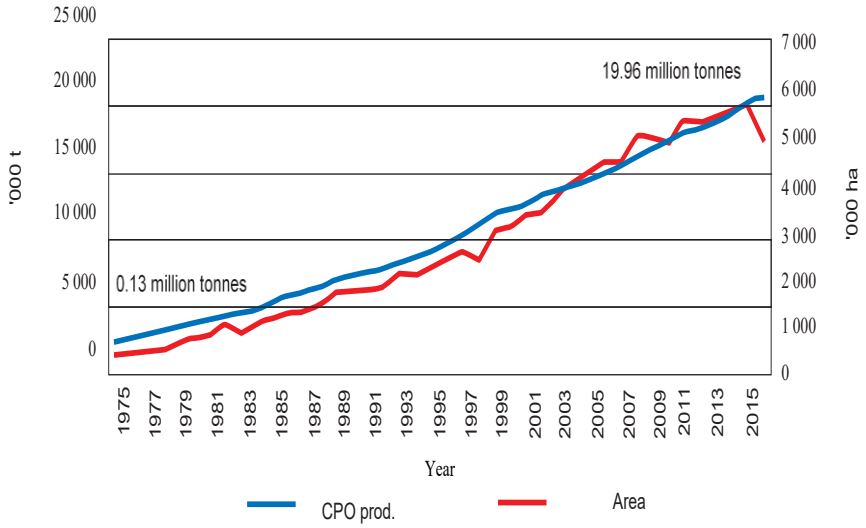


Figure 2.2 Malaysian oil palm planted area and crude palm oil (CPO) production (1975-2016)

Source: (MPOB 2017)

Figure 2.3 Malaysian palm export on crude palm oil (CPO) production (1984-2016)

Source: (UNCTADSTAT 2016)

2.2.2 Export of crude palm oil (CPO)

Malaysian palm oil export experienced a very important development from below 100 000 tons 1960 to 1,605 million tons in 2016. Export of palm oil in 1960 was 90 500 t, with CPO being its primary production over the years (Fold *et al.* 2012). Figure 2.3 shows exports of processed palm oil export (16.05 million tons). South Asia and East Asia have dominated the imports. Malaysia palm products are now exported from Europe and other countries to more than 200 markets globally. Major destinations for exports are India, the EU, China, Pakistan, Egypt and Japan (Ming *et al.* 2002). In 2016, India, the EU, China, Pakistan and Japan remained Malaysia's biggest export market for palm oil.

2.3 Physical characteristics of oil palm plantation in Malaysia

The key varieties used for plantation in Malaysia are tenera, dura and pisifera hybrid due to the high oil yield (Malaysian Palm Oil Council, 2016).

2.3.1 Stage of oil palm

An oil palm plant has different requirements throughout its life stages. In current oil palm industry practice, the plant is divided into three different operational stages. The stages are pre-nursery stage, nursery stage and oil palm plantation stage.

2.3.1.1 Pre-nursery stage

Figure 2.4 shows pre-nursery stage of oil palm starts from seeding, sowing at seedling tray after germinated seeds than pre-nursery seedling transferred to the nursery. The germinated plant is planted with radicle and plumule. The first two leaves and adventurous roots appear in the 2-3 months. The first lanceolate leaf appears one month after planting, along with the first primary root. At the age of four months, the seedling has three to four laminated leaves. With main, secondary and tertiary roots, the root system is well developed. The plant is now autotrophic and prepared for transfer to nursery (Akpo *et al.* 2014).

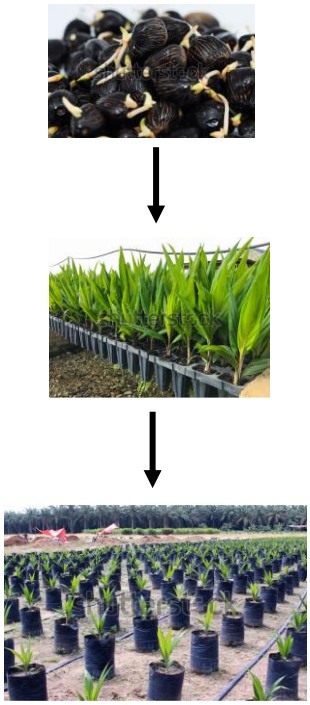


Figure 2.4 Seeding oil palm at nursery stage

2.3.1.2 Main nursery stage

In IOI group (2010), the duration for pre-nursery stage is 0-4 months of the plants age while nursery stage is 4-9 months of the plants age. Furthermore, IOI group (2010) also stressed that the container for the plants must be at least 6 in x 9 in and 12 in x 15 in respectively. Akpo *et al.* 2014) added that a good plant from nursery would have at least 80 cm height, 6 cm root collar diameter, 19 number of leaves and 60 cm^2 area of largest leaf. However, Food and Agriculture Organization of United Nation (2016) suggested that 15-18 number of leaves is sufficient to plant the trees into the field. The good quality plants from nursery stage will ensure effective performance throughout its life. The oil palm nursery stage (4-12 months) has three different leaves which are lanceolate, bifurcate and pinnate. At early stage, the plantlets have lanceolate leaves. Then the leaves started to bifurcate. At the end of the nursery stage, the leaves started to have pinnate morphology (International Plant Nutritional Institute, 2016).

Figure 2.5 Oil palm at main nursery stage

2.3.1.3 Oil palm plantation stage

The genus Elaeis belongs to the Palmae tribe, a member of the group of monocotists under the order Spadiciflorae. The world *Elaeis* originates from the Greek word Elaion, meaning oil, and the term Guinea refers to the origin of the palm on the coast of Guinea (Harlty *et al.* 1988). Table 2.2 shows oil palm characteristics on height, leaf scars, leaves, flower oil, inflorescence, pollination, color fruit, fruit characteristics and mesocarp. Figure 2.6 shows characteristics of oil palm for top, middle and fruit part. Figure 2.7 shows a close-up of an oil palm leaf, while Figure 2.8 shows a male and female inflorescence. Figure 2.9 shows ladder stages of the oil palm fresh fruit bunch (FFB). Figure 2.10 shows chemical contents of the oil palm fresh fruit bunch (FFB) between ripe and unripe.

Table 2.2 Oil palm characteristics

Oil palm	Plant characteristic
Height	60-80 ft
Leaf scars	Spiral
Leaves	25 ft length 200-300 per leaf 3-4 ft long 1.5-2.0 wide
Flower oil	Mononecious Male and female inflorescences in leaf axils
Inflorescence	100-200 branches Spathe 2 weeks prior to anthesis
Pollination	Insects
Color fruit	Green to orange
Fruit characteristic	small plum 10-50 kg bunch bunch - 2000 fruits
Mesocarp	49 % oil 50 % kernel

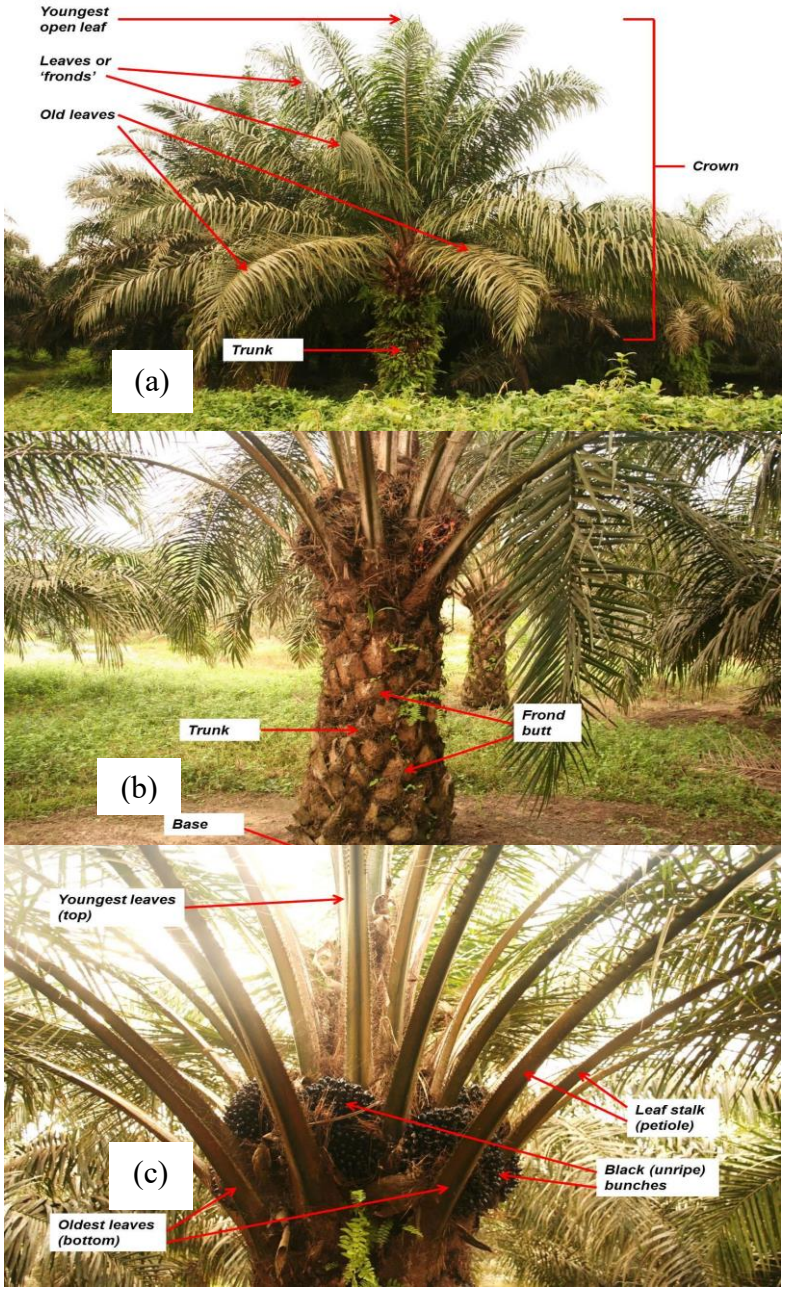
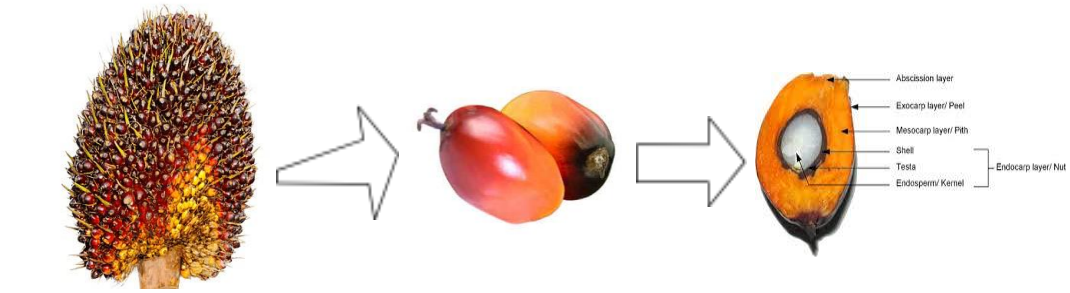


Figure 2.6 Characteristic of oil palm (a) top part (b) middle part (c) fruit

Figure 2.7 Show a close-up of an oil palm leaf

Figure 2.8 Shows a male inflorescence (left) and female (right) inflorescence.

Source: https://www.iscc-system.org/wp-content/uploads/2018/09/Important-terms-Plantation-Maintenance.pdf



Fresh fruit bunch **Fruitlet**

Figure 2.9 Ladder stages of the Oil Palm Fresh Fruit Bunch (FFB)

Source: (Harun *et al.* 2013)

Table 2.3 Characteristic of oil palm fruitlet

Characteristic	Unripe fruitlet	Ripe fruitlet
Constituents	chlorophyll, sterols	carotenoids, triacylglycerols
Age	<12 WAA	16 WAA-20 WAA
Color	Dark purple	Orange red

Source: (Harun *et al.* 2013)

Table 2.4 The ripeness stage of palm oil FFB by Malaysian Palm Oil Board

Image	Stage	Color characteristic
	Unripe	Purplish black
	Under-ripe	Reddish black
	Ripe	Red
	Overripe	Reddish orange

Source: (Harun *et al.* 2013)

Table 2.5 Grading standard used by MPOB

Grading Method	Total Number of Empty Fruitlet Sockets	Mesocarp Color		
		Yellow	Yellowish/Orange	Orange
Number of fruit socket on the bunch	0	Unripe	Unripe	Ripe
	0-10	Unripe	Under-ripe	Ripe
	>10	Unripe	Ripe	Ripe
Number of fruit on the ground	ripe	10%-50% of fruit detached from bunch		
	over-ripe	50%-90% of fruit detached from bunch		
	under ripe	1-9 of fruit detached from bunch		

Source: (Harun *et al.* 2013)

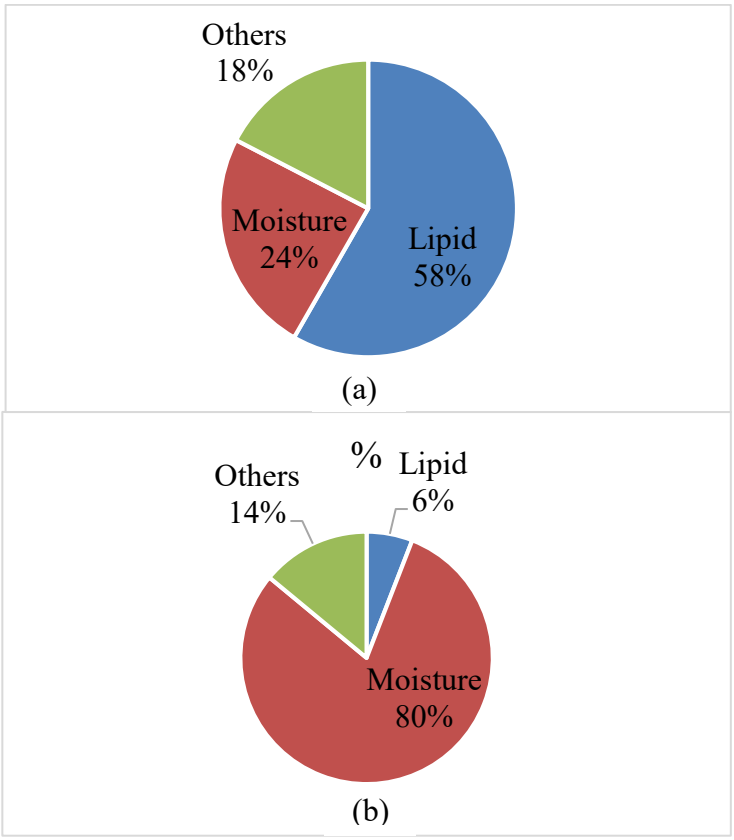


Figure 2.10 Chemical contents of the oil palm fresh fruit bunch (FFB) (a) Ripe (b) Unripe

Source: (Harun *et al.* 2013)

2.3.2 Oil palm plantation management

Three important things to know how much estimated chemical fertilizer would be effectively utilized depends on type of fertilizer, soil and season of the area for oil palm plantation in Malaysia. Inorganic or mineral fertilizer includes urea, rock phosphate (RP), triple super phosphate (TSP), diammonium phosphate (DAP), muriate potash (KCI), kieserite, dolomite and high-grade fertilizer borate (HGFB). Mineral fertilizer is applied by hand to the surface of the soil around the tree (the covered area

corresponds to the cycle of the palm) or sprayed from an air plane over the palms. Urea, TSP and MOP are usually applied twice a year, from February to March and from October to November, while other mineral fertilizers are usually applied once a year. According to those indicator, amount of fertilizer requirement to soil with different group of soil in Table 2.6. In Malaysia we had four types of fertilizers, from first straight fertilizer, wet blends (local compounds), dry blends and complex fertilizer. From those four types of fertilizers, there are some issues that we must understand on application site. Firstly, for straight fertilizer, the issues are on physical quality such as caking and high moisture content. Secondly, wet blends know as local compounds usually more expensive compare another type of fertilizer. Thirdly, for dry-blends (mixtures) segregation of components of different particle size during handling is the main problem in maintaining the declared analysis of dry-blends. And complex fertilizer are important fertilizer. Table 2.6 shows the nutrient input used in maximum exploitation of genetic yield potentials (MEGYP) campaigns (kg $palm^{1}yr^{1}$). Currently, in the commercial farming system, 500 g phosphate rock is applied in the planting hole at the time of planting. Generally, the phosphate is applied in the form of RP at the rate of 1.0-1.2 kg $palm^{-1}$ $year^{-1}$ (Mutert *et al.* 1999). However, Goh and Chew (1995) reported that the annual application of 1.0-1.5 kg PRs $palm^{-1}$ is the best range for peat in Perak. Higher rates of phosphate caused the reduction of Cu and Zn uptake; therefore, it is critical to determine the optimum fertilizer rates for maximum FFB production in the new peat lands. Application of urea, TSP and MOP generally occurs twice a year, from February to March and from October to November, while other mineral fertilizers tend to be applied once per year (Figure 2.5)**.** According to those

indicators, amount of fertilizer requirement to soil with different groups of soil in shown in Table 2.7. From those four types of fertilizers, they have some issues that we must understand on application site. First straight fertilizer, the issues on physical quality example caking and high moisture content. Although during this period of high fertilizer prices, adulteration of fertilizers has been reported in many instances e.g. crushed red bricks for MOP, GML for Kieserite, and even poor-quality ash of unknown origin for bunch ash. Second, wet blends know as local compounds. Different wet-blend granulation plant processes that exists in the country are urea-melt, or steam, or water in the wet phase. Some use pre-mixing before granulation, whilst others do not. Some use batch feeding, some use continuous feeding. All these affect the final quality and consistency of the products. A good optimized process aims to reduce the down time by reducing the off-spec materials that need to be recycled. During peak demand periods if the factory tried to increase output by reducing recycle of off-spec materials the result is often off specification, poor analysis products. Such granular product may appear good physically, but the buyer should always analyze the product for which a high price, often 30-50% higher than straight fertilizers, is paid. For dry-blends (mixtures), segregation of components of different particle size during handling is the main problem in maintaining the declared analysis of dry-blends. It is therefore an inherent problem with such materials even from good reliable suppliers. Nevertheless, analysis of blends from a good supplier can often be better than that of wet-blends in the market. The ways to minimize segregation are to use materials of similar granulometry (*e.g.* granular blends), to minimize the number of components used, and to minimize particle movement within the bags by reducing the free space.

Table 2.6 Nutrient inputs used in maximum exploitation of genetic yield potentials (MEGYP) campaigns (kg palm^{1}yr^{1}) between Indonesia, Malaysia, and Thailand

Nutrient	Application total (1000 t year -1)			Application total (1000 t year -1)[a]			Nutrient removal[b] (kg ha =1 year=1)
	Indonesia	Malaysia	Thailand	Indonesia	Malaysia	Thailand	
Nitrogen(N)	548	374	41	95	91	72	146
Phosphate(P)	61	78	9	11	19	16	19
Potassium(K)	645	821	39	111	199	69	248

[a] The application per hectare was calculated by diving the total application over the oil palm area in 2010 (FAO, 2013)
[b] The final right column shows the nutrient removal, assuming a yield of 30 t fruit bunches ha^{-1} (Corley and Tinker, 2003)

Table 2.7 Nutrient inputs used in Maximum Exploitation of Genetic Yield Potentials (MEGYP) campaigns (kg palm^{1}yr^{1})

Soil Group	N	P^2O^5	K^2O	MgO	B^2O^3	CuO
Ultisols	0.8-1.0	0.55-0.70	2.10-2.50	0.20-0.30	0.05-0.07	–
Oxisols	0.8-1.0	0.75-1.00	1.80-2.20	0.15-0.20	0.05-0.07	–
Inceptisols	0.70-1.2	0.40-0.70	1.20-2.0	0.0-0.15	0.07-0.10	–
Entisols (Sandy)	0.8-1.2	0.50-0.70	2.40-3.00	0.25-0.40	0.70-0.10	0.4
Histosols	0.60-0.80	0.40-0.60	3.00-3.60	–	0.70-0.10	0.6

whether the declared nutrients, especially MgO or P^2O^5 are indeed soluble and plant-available. In such products the straight fertilizer components used may not be soluble or less plant available, e.g. magnesite, non-reactive rock phosphate. Utilization of controlled-release fertilizers and urea-coated fertilizers were invented to reduce ammonia volatilization and act as slow-release fertilizers in the oil palm plantation. Reduced the runoff risks of nutrient loss possibly due to their slow-release properties especially in raining season.

2.3.3 Issues and challenges in oil palm

Table 2.8 shows issues and challenges in oil palm, which are limited arable land, workers, negative allegation on oil palm, and health.

Table 2.8 The issues and challenging in oil palm sector in Malaysia. Limited arable land, workers, negative allegation on oil palm, and health concern on oil palm

Factor	Issues	Reference
Import fertilizer	Malaysia had imported RM 1 144 million worth of chemical fertilizer in 2001	Food and Agriculture Organization of the United Nations (2016)
Limited Arable Land	53% of total agricultural already oil palm plantation planted -10.94 million hectares	(Nambiappan, *et al.* 2018)
Workers	Oversea workers, most of them field in harvesting, manufacturing, weeding and pruning around 39 000 employees in 2016 -CPO rate are RM2500 and RM 3500 -Cost workers for 12 000 employees about RM2.8 billion to RM3.9 billion	(Azman *et al*. 2013).
Negative allegation on Palm Oil	Perception oil palm plantation -unsustainable -pollutes the environment -deforestation -contain 3MCPD ester in product	(Muthiah *et al.* 2017) (Ibrahim *et al.* 2016)
Health Concern on Palm Oil	European Food Standard Authority (EFSA) report 3 MCPD (3-monochoropropane 1, 2 dial) warning of the health	(Clemens *et al.* 2017).

2.4 Utilization of oil palm wastes as organic fertilizer

Another way to get more energy from oil palm planting is by using oil palm biomass more effectively than just palm oil (Figure 2.11). No accurate statistics are available for processing oil palm dry matter. Around 9 kg of dry biomass was produced for every 10 kg of oil palm, only 1 kg was oil palm.

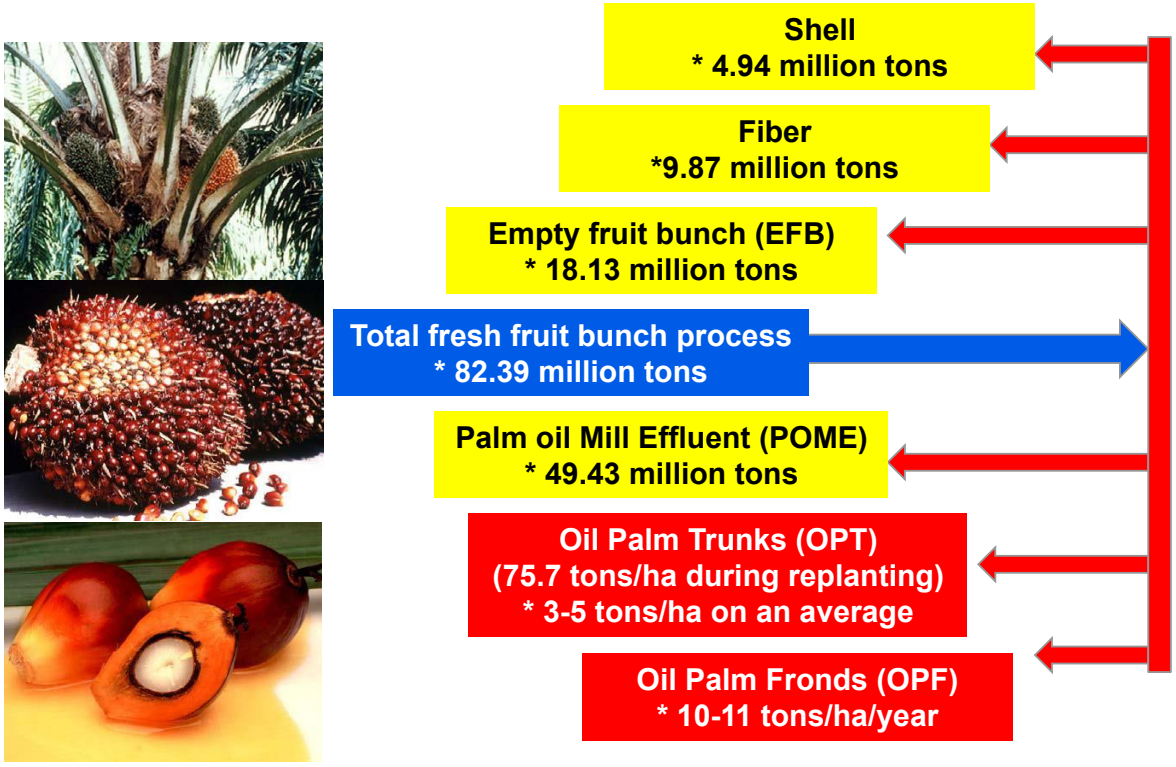


Figure 2.11 Biomass from oil palm plantation at Malaysia

Source: (Biomass Asia Conference Bharathi, 2013)

2.4.1 Empty fruit bunch (EFB)

From Figure 2.12, EFB is obtained after the sterilization and stripping of the FFB. It accounts for about 22% of the volume of wet FFB (Table 2.9). In January 2016, the amount of EFB produced by the palm oil industry is 1,129,835 metric tons based on Malaysian Palm Oil Board (2016). EFB is a by-product from the processing of FFB into crude palm oil (CPO). The amount of EFB produced in January 2020 alone is 1,129,835 metric tons. The EFB previously was burned to before dumping into POME due to its abundance (Zafar 2020). From the characterization, it is noteworthy that the EFB have high potassium % for a biomass (Table 2.10). Therefore, the EFB have a good potential for making high quality compost since P and K easily become insoluble in soil due to oxidation. Although the C:N ratio exceeds the guideline which is 30, studies by (Baharuddin *et al.* 2009) showed that the addition of POME sludge helps the composting process and C:N ratio of 20 can be achieved within 40 days.

Figure 2.12 A male inflorescence (left) and female (right) inflorescence.

Table 2.9 The waste generation by 1 ton of EFB Source:

Products / Residues	Mass for 1 Ton EFB
Palm Oil	220 kg
Palm oil Kernel	60 kg
Empty Fruit Bunches (EFB)	220 kg
Shell	55 kg
Fiber	130 kg
Palm Oil Mill Effluent (POME)	650 kg

Source: (Sarawak Energy, 2016)

Table 2.10 The characteristics of EFB

Component Analysis	Value (mf/wt %)
Cellulose	59.7
Hemicellulose	22.1
Lignin	18.1
Elemental Analysis	**Value (mf/wt %)**
Carbon	48.9
Hydrogen	6.3
Nitrogen	0.7
Sulphur	0.2
Oxygen	36.7
K	2.24
K_2O	3.08-3.65

Source: (Abdullah *et al.* 2011)

2.4.2 Palm oil mill effluent (POME) anaerobic sludge

POME sludge is obtained from all the processes that use water in the palm oil mill (Figure 2.13). However, most of the effluent come from stripping, sterilization, digestion, clarification and pressing. POME sludge production accounts for about 65% from the volume of wet FFB. In January 2016 alone, POME sludge generated by oil palm industry was about 3,338,148 metric tons (Malaysian Palm Oil Council, 2016). It has been estimated that 5 - 7.5 tons of water are required to produce 1 ton of crude palm oil and more than 50% of the water ends up as palm oil mill effluent (POME). Sterilization of FFB, clarification of the extracted CPO, hydrocyclone separation of cracked mixture of kernel and shell hydrocyclone contribute about 36, 60 and 4% of POME respectively in the mills. In Malaysia about 53 million POMES is produced every year based on palm oil production in 2005 of 14.8 million tons. It is estimated that about 0.5- 0.75 tons of POME will be discharged from mill for every ton of fresh fruit bunch. Typically, palm oil mill wastewater is low in pH because of the organic acids produced in the fermentation process, ranging about 4-5. It also contains large amounts of total solids (40,500mg/l), oil and grease (4000 mg/l) (Ma, 2000). Wastewater includes dissolved constituents such as high concentration of protein, carbohydrate, nitrogenous compounds, lipids and minerals, which may be converted into useful materials using microbial processes.

Table 2.11 shows the parameters in POME above far exceed the regulations based on Environmental Quality Regulations set by Department of Environment. If released

raw, high BOD will drastically decrease DO and kill all aquatic animals, low pH leads to gills diseases and kills all aquatic animals, presence of solids will make the discharged area severely contaminated and high ammoniacal nitrogen is toxic to aquatic animal which also leads to death of aquatic animals. However, the microbiome in POME sludge can be proven useful in composting technology. The high diversity of the microbiome with high respiration activity due to excessive substrate present in POME sludge leads to the ideal utilization in composting, acting as both inoculant and moistening agents for biomass (Baharuddin *et al.* 2009*).*

Figure 2.13 POME anaerobic sludge on the pond

Source: (Nyakuma, 2019)

Table 2.11 The characteristics of POME Sludge

Parameter	Mean	Range	Discharge Regulation
pH	4.2	3.4 – 5.2	5-9
Biological Oxygen Demand (mg/ml)	25000	10250 – 43750	100
Chemical Oxygen Demand (mg/ml)	51000	15000 – 100000	-
Total Solids (mg/ml)	40000	11500 – 79000	-
Suspended Solids (mg/ml)	18000	5000 – 54000	400
Volatile Solids (mg/ml)	34000	9000 – 72000	-
Oil and Grease (mg/ml)	6000	130 – 18000	50
Ammoniacal Nitrogen (mg/ml)	35	4 – 80	100
Total Nitrogen (mg/ml)	750	180 – 1400	-

Source: (MPOB, 2016)

2.4.3 Potential use of empty fruit bunch (EFB)

Composting of EFB is an alternative technique in converting waste to valuable products. Empty fruit bunch is a common raw material used in composting. EFB is considered suitable for the production of good quality compost due to its fibrous characteristic (Suhaimi *et al.* 2001). The application of EFB in soil offer benefits in the longer term for the physical-chemical properties of the soil, and more generally for soil fertility (Wingkis *et al.* 1998). Empty fruit brunch is mostly used as a substitute for mineral fertilizer by direct application in the field, after incineration or after composting. Application of fresh EFB to the plants returns mineral nutrients and organic matter to the soil and helps to maintain soil fertility. Plants also benefited from the positive effect of EFB through increased nutrient uptake and yield (Zaharah *et al.* 2000). It is also resulted in improved plant growth and yield. Addition of 150 g compost of EFB per bag singly enhanced seedling and development, and improved yield by 71% comparable to control palms (Aisueni *et al*. 2001). Increased vegetable crop production and yield were recorded by addition of EFB compost in soilless system (Ismail *et al.* 2004). Various composts containing EFB are being produced in Malaysia production. The nutrient values of compost vary depending on the content of the base materials used. Higher ratio of fibrous materials used in the compost resulted in low nitrogen content (Suhaimi *et al.,* 2001). EFB which is fibrous, containing about 30% dry matter, 2.5% cellulose and 63% water is often mixed with other materials, particularly chicken manure and POME in compost production.

2.4.4 Incorporation between EFB and POME anaerobic sludge

Compost is a process of converting organic waste into rich soil-like material. Composting technology is similar to solid state fermentation. Therefore, the requirement is similar to solid state fermentation including the need of a semi-moist solid medium and inoculum. According to Oviasogie *et al.* (2013), for 1 tonne FFB processed, an oil palm mill generates 230-250 kg of EFB, 130-150 kg of fibre, 60-65 kg of shell and 55-60 kg of kernel (Sarawak Energy, 2016). In addition, for 1 tonne FFB processed, 600 m^3 of POME is generated. Since the volume of the industry is big, composting technology is a viable solution for many reasons. First, the composting technology will reduce the waste generated. Specifically, for EFB and POME, Singh et al (2010) stated that the reduction will be up to 75% and 20% respectively. The property of the compost may be beneficial to the plant as soil conditioner and soil fertilizer. The mixed dosage of compost and chemical fertilizer could produce the best plant physical performance as reported by Chiew *et al.* (2013). The process for production of compost in oil palm industry, preferably using EFB, can be referred to in Bello *et al.* (2014). In the report, to produce the best compost, EFB is best chopped into smaller pieces prior composting. The chopping will make the total surface area/volume (tsa/v) ratio bigger and thus, exposing more surface area for the microorganisms to live and metabolize the raw material. The composting begins by inoculating the raw materials with POME sludge. POME sludge has two roles in composting. First, the POME sludge serves as an inoculum source. Second, POME sludge moistens the EFB and make it a semi-moist medium. As the metabolism occur, the temperature will increase and dry out the POME sludge. Therefore, Bello *et al.*

(2014), suggested that the compost is turned and POME sludge is added if the moisture of the EFB reached 60%. The compost is considered as mature once the ratio of C/N reach <30. Once the C/N ratio reach 40, the turning and moisten of compost is stopped and the microbes are left to use up the moisture in its metabolism. Although the initial C/N ratio of EFB is relatively high which is 70, the addition of POME sludge greatly aids the metabolism of the compost and enables the C/N ratio to be reduced as low as 20 in 40 days with proper turning since aerobic metabolism is faster than anaerobic metabolism and the moisture is kept at 60-70% (Baharuddin *et al.* 2009). The microbial profile in compost changed in succession and different from stage to stage. Studies by Baharuddin *et al.* (2009) using DGGE showed that different dominant phyla were detected throughout the composting process by EFB and POME sludge. In the beginning, *Cyanobacteria, Delta-proteobacterium* and *Firmicutes* are found to be dominant. *Firmicutes* was one of the dominant groups because it is found in abundance in EFB. Then, as the compost enter the thermophilic stage, *Cyanobacteria, Delta-proteobacterium* and uncultured *Desulfobacteraceae* was found to be dominant. Then, as the compost enter the pre-mature stage, uncultured *Chloroflexi* and *Proteobacterium* was found to be the dominant group. Lastly, as the compost matures, only uncultured *Chloroflexi* was found to be dominant. In Mohd Zainudin *et al.* (2014), specific genus of bacteria had been identified for its different function. Throughout the compost, *Bacillus sp.* was found out to be the most dominant group. In the study, it was found that *Solibacillus silvestris* was the main bacteria responsible for lignocellulosic degradation. Then, as the degraded, simpler material are readily available, *Lysinibacillus massiliensis* becomes dominant especially during

thermophilic phase of the composting process. There are more dominant bacteria throughout the process but the function was not confirmed. Therefore, with the aid and succession of different bacteria, a high-quality compost can be produced successfully.

2.4.5 Environmentally friendly features in the processing of compost

An average oil palm mill can handle about 700-1000 metric tons (mt) of fresh fruit bunch daily (www.globalfeldaventurev.com, 2014). At the mills where oil extraction takes place, solid residues and liquid wastes are generated. Palm oil production is a major agricultural industry in Malaysia, in which palm oil mill effluent (POME) and oil palm empty fruit bunch (EFB) are considered as major waste products from the palm oil industry. These waste products create an environmental hazard and entail high disposal costs every year. Composting is a biologically based process, which is practiced in order to stabilize the organic matter for soil amendment (compost) and to protect the environment from the detrimental effects of these waste products. Empty fruit bunch (EFB) and palm oil mill effluent (POME) used as compost provided a nutrient source and soil conditioner. To minimize pollution, a new usage for these wastes ought to be looked into.

2.4.6 Plant uptake on soil applied with compost

Using compost from organic wastes is much cheaper than the fertilizer produced in the industry. Compost also improves the soil water holding capacity and provides better tilts. The use of compost is no longer limited to its use as a soil amendment. Compost technologies are emerging rapidly valuable tools in pollution prevention and control.

Compost is now being used in erosion control on highways, the clean-up of contaminants in storm water runoff and in the remediation of soils contaminated with heavy metals or toxic organic compounds. With regards to the concerns about global warming, composting is playing a major role. The organic decomposition of wastes in anaerobic landfills and open lagoons has methane as the major product, while the same process forms CO^2 in anaerobic composting. Since methane has 22 times compared to carbon dioxide impact on global warming, composting materials abates this problem (Baharuddin *et al.* 2010).

2.4.7 Compost as slow-release fertilizer

Almost one generation of oil palm plantation in Malaysia used 100% inorganic fertilizer that caused infertility of soil and high cost of import inorganic fertilizer in oil palm plantation very year. Use of organic fertilizer from oil palm biomass in Malaysia at 10% fertilizer equivalent can reduce 1.0 billion Ringgit of fertilizer import. Increased fertility of soil can be expected, because compost is high in organic matter and promoted growth of beneficial microorganisms in soils. There is also increased infiltration and permeability of heavy soils, thus reducing erosion and runoff. It also resulted in improved water holding capacity, thus reducing water loss and leaching in sandy soils. Other advantages are improved cation exchange capacity (CEC) of soils and growing media, thus improving their ability to hold nutrients for plant use, as well as improving and stabilizing soil pH.

2.5 Soil Microbes

Recently, soil microbial profile is a subject of interest for oil palm industry. By using DGGE, Asakawa *et al.* (2008) stated that phyla *Firmicutes* and *Chloroflexi* are the dominant groups in topsoil followed by *Proteobacterium.* DGGE was used in many research studies to detect the shift in microbial profile of a system (Wallis *et al.* 2010). The relationship between the utilization of chemical fertilizer towards soil fertility is inversely proportional. Candidi *et al.* (2019) stated that utilization of chemical fertilizer will result in decreased soil biomass, soil respiration, soil enzymatic activities and soil buffering ability. To relate this, another research by Chaporro *et al.* (2012) discovered that a plant can select a subset of desired microbes by releasing a combination of low molecular weight substrates and phytochemicals at its root and thus forming beneficial rhizhosphere microbiome to the plant. Furthermore, the plant released a concoction of hormones, low molecular weight nutrients and certain phytochemicals only by induction. For example, if the plant is lacking in N, then it will release the concoction for attracting N-fixing bacteria. With the addition of synthetic nutrient to the soil via chemical fertilizer, the concoction is not released and in prolonged time, the bacterial respiration will decrease. This is why (International Foundation for Organic Agriculture, 2016) strongly suggested that organic farming must be progressively researched in order to discover its full potential on agriculture. Various studies have been done on the effect of compost to oil palm physical growth. However, there were no studies yet on the effect of mixing compost and soil to microbial profile of the mixture. Therefore, this study tries to fill in the gap

of the current research on the microbial profile for the addition of compost to the soil microbial profile. Table 2.12 shows the techniques that can be used in microbial diversity.

Table 2.12 Advantages and disadvantages between DGGE and Illumina MiSeq techniques

Techniques	Outline of methods	Advantage	Disadvantage
DGGE[a]	-Target 16S rRNA sequence (200-600basepair) -Fingerprinting of community profile -Phylogenetic affiliation	- Rapid and simple monitoring of community shift over time - The band intensity offers overview of dominant species	Limited resolution in complex profile - Less sensitivity - Not quantitative
Illuminia MiSeq[b]	- Read length sequence of 50 - 250bp - Reads per run up to 3 billion	High throughput - High yield of sequencing – Time consuming	Equipment very costly

[a] Muyer *et al.* 2004
[b] Cho *et al.* 2017

Table 2.13 Bacteria reaction depending on types fertilizer

Wheat and Rice	Element Effect	Bacteria	Cited
Chemical Fertilizer	-application N lead acidification soil - the action of ammonium oxidizing bacteria and archaea that produce protons - High Acidobacteria - Low Proteobacteria	*Archea* -phyla *Chloroflexi* & *Thaumarchaeota* -class *Anaerolineae* & *Nitrosophaerales* *Increases of* - *Gemmatimonadetes phylum, class,order*	Guo *et al.* 2010
Chemical Fertilizer (CF) with (OIMF)	-Increase abundance of some generally oligotrophic bacteria. -Slow growing properties as typical of a K-selected life strategy -abundant in soils with low recourse availability and low organic C conditions and it is negatively correlated with soil pH - OIMF treatment improved soil ammonium and nitrate content, effect ammonia oxidizing archeal *Thaumarchaeota*	*Acidobacteria* *Increases* *-Acidobacteria & Gemmatimonadetes*	Spang *et al.* 2010; Pester *et al.* 2011 Fierer *et al.* 2007
Organic Fertilizer (OF)	-More copiotrophic - including the alpha and Betta subclasses, - with higher abundances nutrient rich high C soils - Increase soil organic C and N	*Proteobacteria (Higher- `subclass` Alphaprotobacteria & Betaprotobacteria)* *Increases* - *Betaproteobacteria*	Fierer *et al.* 2007; Newton and McMahon, 2011; Zhou *et al.* 2015.

CHAPTER 3

GENERAL MATERIALS AND METHODS

3.1 Plant growth

The growth of the seedlings was monitored at three, six and eight months after transplanting by recording the girth size, plant height, frond number, frond length and dry weight. The greenness of the oil palm seedling leaves or the chlorophyll content was measured by using a SPAD 502 Chlorophyll meter. The planting media were sent to the laboratory to be analyzed for their nutrient content at the beginning and at the end of the experiment. Leaf samples were harvested from frond number 3 for nutrient analysis at the end of the study by drying the sampled leaves. For biomass determination, the fronds and the girths were separated and dried at 70^0C in an oven until constant weight.

3.2 Soil properties and nutrient content

Soil having weight of 20 g was placed into a plastic bottle. Distilled water was added into the bottle. The soil was shaken intermittently for one hour and left to stand overnight. The pH was calibrated by using buffers of pH 4.00 and pH 7.00. Basic exchangeable cations were extracted and determined by electrolyte solution in 0.01 M KCl. CEC was determined with ammonium acetate and the determination of ammonium ions in the soil was done using the colorimetric method. The total organic C content of the soil was determined by using the Walkley and Black titration method (Gelman *et al.* 2012). The total N content of the soil was determined using alkaline

phenol and hypochlorite. In order to measure the P content, the soil sample was first digested for 1 ¾ hours by using a Block Digester at 200 ºC and the analysis of P was then done using the Auto-Analyser. Determination of soil exchangeable cations (K, Ca, Mg, and Na) was done by using 1M ammonium acetate. The potassium, magnesium and calcium determinations were done on the AAS by pipetting 2 ml of the original solution and adding 20 ml of 825 ppm Strontium nitrate by using the Auto-Diluter 111. The sodium determination was done using the original solution and reading on the AAS (Sime Darby Research, 2018).

3.3 Microbial analysis

DNA extraction from water was done by DNA extraction was carried out using the POWERSOIL^{TM} Sterivex^{TM}DNA Isolation Kit following manufacturer`s instrument (Mo Bio Laboratories, Carlsbad, CA, USA). Table 3. 1 show, method for High-throughput 16S rRNA sequencing.

3.4 Statistical analysis

Data for soil properties and nutrient depletion were identified by analysis of variance (ANOVA) using the SAS Software Windows Version 9.1 (SAS, 2007). Turkey analysis at $p \leq 0.05$ was used to test significant difference between the treatments. Tukey's honestly significantly different test for all pairwise comparisons were calculated after ANOVA to compare treatment means. Statistical analysis of DGGE profiles, using Gel images of the DGGE profiles were converted, normalized and digitized using Quantity One 3.0 software (Bio-Rad).

Table 3. 1 Method for High-throughput 16S rRNA sequencing

Step	Method	Material / Equitment
1	Extracted DNA	16S rRNA gene V4-V5 region
2	Primer	515F (5′-GTGCCAGCMGCCGCGG-3′) - forword reverse primer 907R (5′-CCGTCAATTCMTTTRAGTTT-3′) - reverse
3	PCR	25 ul - sample 10x Taq buffer and Taq polymerase (BioLabs), 20 uM of each primer 2 mM of each dNTP 25 mM $MgSO_4$ (Toyobo) 50 ng of cDNA
4	Purified PCR	Qubit dsDNA HS Assay Kit (Life Technologies, Oregon, (USA)
5	MiSeq Sequencing System	Nextera XT DNA Library Preparation Kit
6	Analysis MiSeq	QIIME v.1.9.0 i. raw paired-end reads were assembled using PANDAseq tool ii. trimming process to remove low quality and ambiguous reads iii. Operational taxonomic units (OTUs) with 97% sequence similarity using the de novo OTU picking pipeline iv. UCLUST v1.2.22q to classify each representative sequence prior aligning the sequences v. rarefied OTU tables - alpha diversity measurement, and rarefaction curves - Shannon diversity metric vi. Beta diversity - principal coordinate analysis (PCoA) and cluster analysis - Jackknife beta-diversity and UPGMA

Source: (Mohd-Nor *et al.* 2018)

3.5 Thesis overview

Figure 3.1 show overall research methodology on objective one, objective two and objective three. Chapter 1 of this thesis introduces the problem statements and objectives of this research. Chapter 2 addresses other researchers' findings, with some discussion, including a review on oil palm plantation recovery on secondary forest, oil palm at nursery stage, production of inorganic fertilizer (compost) as slow release fertilizer, and planting oil palm plantation five years checking on chemical, biological, physical characteristic, microbial, oil extraction and economic statically analysis. Chapter 3 covers general materials of the research. This includes the first objective, second objective and third objective. Chapter 4 compares different nutrient content and microbial diversity on secondary forest soil and after 25 years of application of chemical fertilizer in oil palm plantation. In Chapter 5, in to improve soil fertility, it was proposed to apply compost from EFB and POME anaerobic sludge, in addition and for partial replacement of inorganic fertilizer. The effect on soil physical characteristics, soil physiochemical, microbial diversity and oil extraction were measured and assessed. Chapter 6 is on the nursery study using different combinations of inorganic fertilizer and compost. In Chapter 7, the summary and conclusion of this study were presented. Recommendations for future work were also suggested.

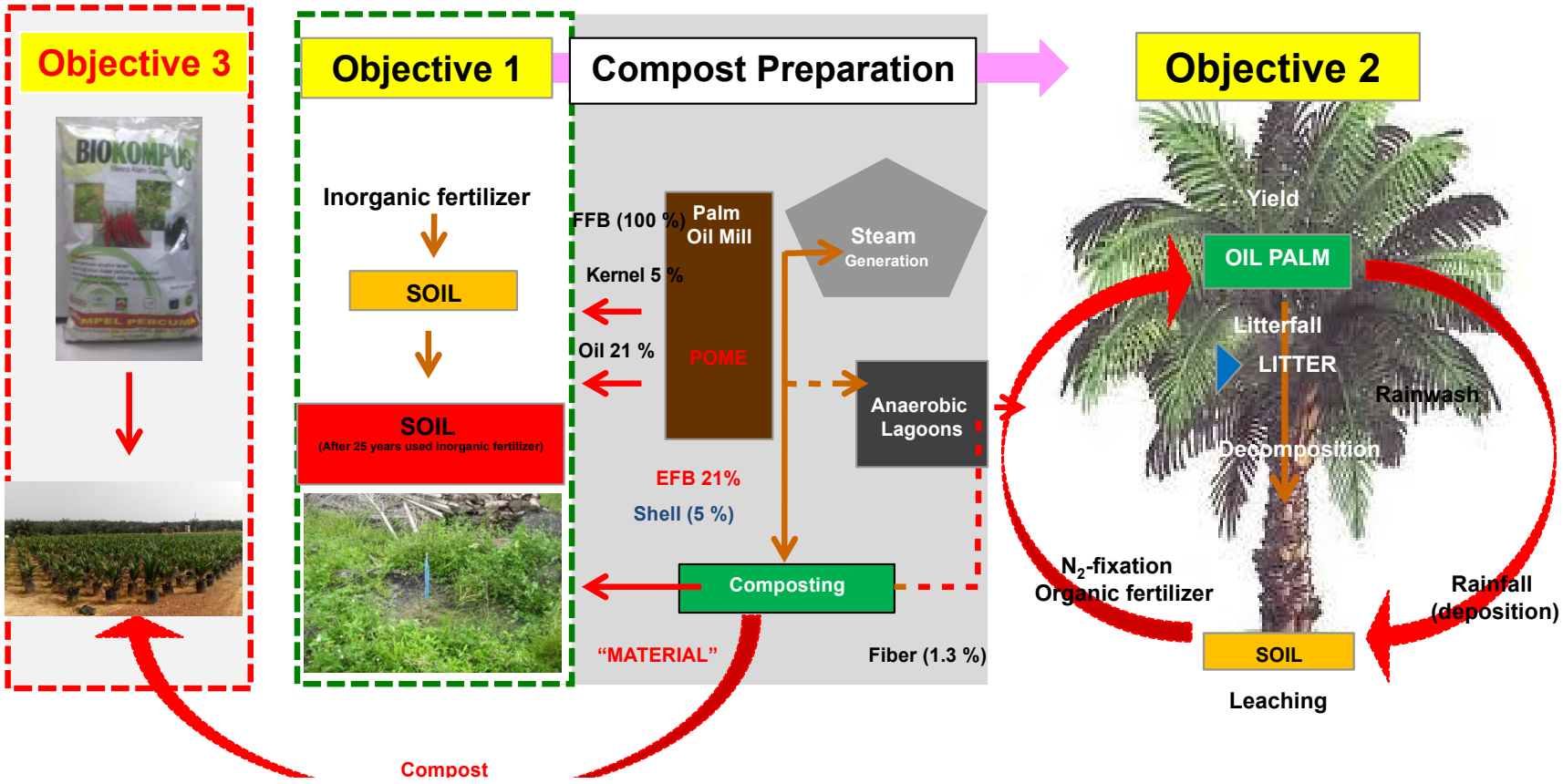


Figure 3.1 General experimental layout of this study objective 1, objective 2, and objective 3

CHAPTER 4

EFFECT OF INORGANIC FERTILIZER APPLICATION ON SOIL BIODIVERSITY IN OIL PALM PLANTATION

4.1 Introduction

Soil conservation is strongly linked to the soil as a habitat for the development of a wide variety of species and is directly associated with the broader biosphere comprising plants, animals, microorganisms and the habitat. Biological activity is a primary factor in soil formation in both physical and chemical terms. Soil microbial organisms have important responsibility for soil functionality and protection of the environment. Several studies have shown that soil microbial population profile and composition are important determinants of soil health and can be influenced by various agricultural management variables, including repeated application of fertilizers, pesticides, crop rotation, tillage and machine usage (Dorr *et al.* 2012). Continuous long-term cultivation usually results in plant growth inhibition and bad soil borne diseases that have been identified in continuous cropping (Yang *et al.* 2012 and Liu *et al.,* 2014). Continuous cropping system is commonly practiced in the oil palm plantation. Different factors were regarded to minimize continuous crop barriers, including physicochemical deterrent of soil and plant-borne pathogens (Fuentes *et al.* 2009; Huang *et al.* 2013). The microbial disturbance and the connection between these effects and soil productivity remained unclear (Wang *et al.* 2019). Very few studies have recorded the differences in soil microbial populations in continuous cropping systems for oil palm plantations and their impact on soil micro flora and oil palm

plantation. Bacteria is the most complex and prolific soil microorganism (Gans *et al.* 2005). Bacterial groups can be the key of plant growth promoters and agents of soil borne pathogens (Rumberger *et al.* 2007). Their effects have led to costly, laborious and time-consuming weakness in microbial research (Roesch *et al.* 2007). New technology known as next–generation sequencing (NGS) technologies detect deeper insight of soil bacterial communities. In this study, four replications in oil palm plantation replanted after the first 25 years cycle planting history in Negeri Sembilan Malaysia were selected to investigate the effect of long-term continuous cropping on soil physiochemical, bacterial abundance, and bacterial community structures. The objective of this study was (1) to explore potential correlation between soil physiochemical properties and soil fertility as compared to forest soil; and (2) to elucidate the changes in soil bacterial communities on oil palm plantation under continuous cropping system for one plantation cycle.

4.2 Materials and methods

4.2.1 Description of the study site

The soil samples were collected from the field site situated in enrichment planting (55º 40'S, 12º 18'E) and secondary forest (3.02471^0 E 102.36846) at FELDA Agricultural Services Sdn Bhd, Serting Hilir, Negeri Sembilan, Malaysia. The soils in this study area were classified as cullovium at the base of hills and ridges. It has been observed that the soils are not sedentary but have moved down from above and accumulated at the base of the hills and ridges. The terrain is normally hilly to steep where these soils occur and the underlying geology usually consists of intercalated phyllites, quartzites

and shales. Soil samples were collected in January 2014 after running the field experiment previously planted with 25 years of oil palm. The sampling time ensured that there were minimal effects of the most recent fertiliser schedule from FELDA, and that possible effects observed would be was left idle to undergo natural regeneration without ant reforestation activity at Jalan Kampung Lui Selatang Serting Hilir, Negeri Sembilan.

4.2.2 Soil properties and nutrient

The pH was determined by electrode with distilled water between samples. The settings of the pH meter were calibrated with buffer solutions before, during and again at the end of a run of samples. The soil sample (20 g) was mixed with 50 ml distilled water and left overnight prior to soil pH determination (MS, 1980). Total nitrogen and total phosphorus of soil samples were determined using Block-Digester and Auto-Analyser according to SIRIM standard method for soil chemical analysis (MS, 1980). The total organic carbon content of the soils was determined using Walkely and Black titration method (Gelman *et al.* 2012). Cation exchange capacity (CEC) was determined by leaching the soil samples with ammonium acetate using colorimetric method (Sikora *et al.* 1990). The analysis of soil exchangeable cation (K, Ca, Mg, and Al) was conducted by leaching the soil samples using 1 M Ammonium acetate pH 7 (**Section 3.4.2**).

4.2.3 Denaturing gradient gel electrophoresis (DGGE)

Total genomic DNA was extracted from 10 g soil carried out with a MoBio Power Soil DNA Isolation Kit (Mo Bio Laboraries Inc., carlsbad, Ca, USA) following the manufacturer's protocol. Total DNA quality and concentration were measured by the Nano Drop ND-2000 spectrophotometry (Nano Drop Techhnologies, Thermo Scientific, USA) and run gel 1% using electrophoresis. The PCR product was loaded onto a 1.5-mm-thick vertical denaturing gel containing 8% acrylamide with a gradient from 30% to 60%. One hundred percent of the denaturant corresponded to 7 M urea and 40% (v/v) formamide. Electrophoresis was performed at 200 V at 60°Cfor 5 h. After electrophoresis, the gels were stained with gel red (1 mg/L) and viewed with the Gel Doc XR+ System (Biorad laboratories, USA). Phylogenetic tree analysis of DGGE bands was conducted based on clustering method applied on the distance matrix computed using Unweight Pair Group method with Arithmetic Mean (UPGMA) in the PyElph Software.

4.2.4 High-throughput 16S rRNA sequencing

The high-throughput 16S rRNA sequencing method as described in **Section 3.3.**

4.2.5 Sequencing data analyses

High-throughput MiSeq data was processed and analysed using QIIME v.1.9.0. The raw paired-end reads were assembled using PANDA seq tool, followed by a trimming process to remove low quality and ambiguous reads. The high-quality reads were clustered into operational taxonomic units (OTUs) with 97% sequence similarity using

the de novo OTU picking pipeline. UCLUST v1.2.22q was used to classify each representative sequence prior aligning the sequences against Green genes database v13.8 using the PyNAST program. The rarefied OTU tables were used as the basis for the alpha diversity measurement, and rarefaction curves were computed using the Shannon diversity metric. Beta diversity was analysed using principal coordinate analysis (PCoA) and cluster analysis using Jackknife beta-diversity and UPGMA, respectively.

4.2.6 Statistical analysis

Data for soil properties and nutrient depletion were identified by analysis of variance (ANOVA) using the SAS Software Windows Version 8 (SAS, 2001) **(Section 3.4).** Statistical analysis of DGGE profiles, using Gel images of the DGGE profiles were converted, normalized and digitized using Quantity One 3.0 software (Bio-Rad).

4.3 Results and discussion

4.3.1 Soil chemical properties

The effect of inorganic fertilizer after 25 years application on oil palm plantation is shown in Table 4.1, whereby pH and cation exchange capacity (CEC) were significantly different between plot at $p<0.05$. Samples at Plot P1, P2, P3, and P4 with two different depths 0-15 cm and 15-30 cm at fertilizer area and unfertilized area was the same at pH 4.5, but at secondary forest soil the value of pH was 4.6. The electrical charge of some of the soil components that contributed to the CEC is affected by the pH of the soil. These components have an OH group on their edges. It is known that OH group can release or absorb protons. At high pH, protons are released from these groups, their charge becomes negative and, as a result, CEC of the soil increased (http://www.smart-fertilizer.com/articles/Cation-Exchange-Capacity). The result of CEC showed significantly increase when pH value decreased, secondary forest soil pH value was 3.50 compared with Plot 1 to Plot 4 with different depths, with pH around 4.75 until 5.83. CEC refers to the amount of negative charges available on the surface of soil particles. It gives an indication of the potential of the soil to hold plant nutrients, by estimating the capacity of the soil to retain cations, which are positively charged substances. Therefore, the CEC of the soil directly affects the amount and frequency of fertilizer application.

Furthermore, in Table 4.1 the macronutrient after 25 years applied inorganic fertilizer were significantly different at $p<0.05$. It also showed a decrease after 25 years' application: inorganic fertilizer from 0.12 % to 0.03-0.05 % total nitrogen (N), 2.52 %

to 0.18-1.05 % organic carbon, 0.28 % to 0.09-0.12 % K, 2.34 % to 1.43-1.77 % boron, and 5.42 % to 1.35 % – 3.13 % and manganese other element increased after 25 years inorganic fertilizer application such as CEC (3.50 % to 4.75 – 5.81 %), avail P (3.13 % to 4.75 – 5.83 %), calcium (0.76 % to 1.04 – 1.24 %), magnesium (0.22 % to 0.26 – 0.40 %), aluminium (0.42 % to 0.38 – 0.45 %), Mangan (60.54 – 177.59 %), ferum (311.41 % to 708.38 – 8.64.9 %), zinc (1.77 % to 1.73 – 1.85 %) and silica (19.93 % to 53.45 – 120.23 %). Total nitrogen, at secondary forest 0.12 % and after 25 year application inorganic fertilizer the value decreased until 0.03-0.05 % respectively. Data on macronutrient, available P, Ca and Mg showed secondary forest soil with higher valued and decreased after 25 year inorganic fertilizer application. Secondary forest soil on available P, 3.13 mg/kg after 25 years inorganic fertilizer application increases between 4.75-5.83 mg/kg. On Ca, 0.76 exch cmol(+)/kg on secondary forest soil and 1.04-1.24 exch cmol(+)/kg after 25 years application inorganic fertilizer. Values on Mg at secondary forest soil 0.22 exch cmol(+)/kg increased between 0.26-0.42 exch cmol(+)/kg after 25 years inorganic fertilizer application. Data on K and Al showed different pattern with secondary forest soil high value compared with soil after 25 years inorganic fertilizer. Data on K at secondary forest soil 0.28 mg/kg, after 25 years application inorganic fertilizer the valued decreased between 0.09-0.12 mg/kg and Al from 0.42 exch cmol(+)/kg to 0.31 exch cmol(+)/kg. Table 4.2 shows micronutrients Boron (B), Ferum (F), Mangan (Mn^{2+}), Zinc (Z), Silica (Si) and Mangan $(Mo)^{+}$ contents were significantly different only different between plots. Data pattern for secondary forest soil for Mn (60.54 mg/kg), Fe (311.41 mg/kg), Zn (1.77 mg/kg) and Si (19.93 mg/kg) are low and increased after applied 25 years of inorganic

fertilizer to 177.59-187.85 mg/kg (Mn), 689.22-864.9 mg/kg (Fe), 1.73-185 mg/kg (Zn) and 53.45 mg/kg (mg/kg). Another micronutrients B and Mo in different pattern valued on secondary forest data 2.43 mg/kg and 5.42 mg/kg decrease value after applied 25 years inorganic fertilizer at soil 1.43-1.77 mg/kg (B) and 1.25-3.13 mg/kg (Mo) mg/kg.

4.3.2 Soil distribution

In order to investigate the effect of inorganic fertiliser to the soil structure, particle size distribution of different samples were studied (Table 4.2). The results show that the soil properties (clay, slit, course sand, and fine sand) in particle size distribution are significantly different within plots and secondary forest soil. The results showed application of inorganic fertiliser the structure of on particle size distribution on clay (43.65 %) and coarse sand (2.49 %) and increased percentage after application of inorganic fertilizer frequently to clay (45.98 - 48.10 %) and coarse sand (11.26 - 14.48 %). Particle size distribution on silt decreased from 38.00 % (secondary forest soil) to 25.5 % after 25 years inorganic fertilizer and 26.63 % to 11.37 % for fine sand.

4.3.3 Denaturing gradient gel electrophoresis (DGGE)

The denaturing gradient gel electrophoresis profiles for the plots after 25 years application of inorganic fertilizer and forest soil is shown in Figure 4.1. It clearly shows that samples from plot after 25 years applied inorganic fertilizer and secondary forest soil was distinguished by smear the separated band except on forest soil at depth 0-15 cm (number 5) with thick band pattern. Figure 1B, second DGGE based sample

from first DGGE on secondary forest at 0-15 cm depth on thick band soil (a, b, c, d, e, f, g, h, and i) microbial profile dominant for secondary forest soil at 0-15cm compared to plot with inorganic fertilizer after 25 years applied at soil for the analysis taken from at different points in different plots. Therefore, the effect of the fertilizer application gave an impact on the diversity of the microorganisms in soil of secondary forest soil. Soil from oil palm plantation applied with inorganic fertilizer at fertilized and unfertilized area with 0-15 cm and 15-30 cm depths showed were heterogeneous which means both treatments affected the microbial diversity in soil. Detailed analysis of each sample showed the predominance of different microbial species in each community. Different environment conditions and substrate characteristic had contributed to the species of certain dominant microbes throughout plot after 25 years applied inorganic fertilizer and secondary forest. In this study, the phylogenetic relationship shows phyla with *Acinobacteria, Firmucitus* and *Proteobacteria in* Figure 4.2.

4.3.4 Community composition of bacteria

Extracted soil from 10 g used MoBio Power Soil DNA Isolation Kit than run High-throughput 16S rRNA sequencing and analysis data using QIIME v.1.9.0. After quality filtering and singleton OTUs were gained from the 5 sample with the pyrosequencing based analysis Figure 4.3, *Acidobacteria Proteobacteria, Bacteridetes, Actinobacteria, Planctomycetes, Firmucutes*, and *Chloroflexi* were the dominant bacterial phyla (relative abundance>1%). Additionally, the relative abundance of *Bacteroides* and *Firmicutes* phyla decreased with long term continuous cropping of oil palm plantation. Compared to the soil after 25 years inorganic fertilizer application, secondary forest

soil had higher relative abundance of *Acidobacteria* and *Chloroflexi* and lower abundance of *Proteobacteria*. Furthermore, comparison of the relative abundance of the top 20 classified bacterial genera showed significant variation among secondary forest soil after 25 years application inorganic fertilizer (Figure 5). Table 4.3 shows that *Archaea* was present at secondary forest but not after 25 years applied inorganic fertilizer.

4.3.5 Correlation of bacteria

Understanding the correlation between soil properties and microbial diversity is a key to the sustainable productivity in oil palm plantation as continuously cropping system in Malaysia. It has been reported that the application of inorganic fertilizer decreased soil pH. In this study, the soil pH in secondary forest soil was pH 4.6. The soil pH after 25 years application of inorganic fertilizer decreased to only pH 4.5. Repeated application of inorganic fertiliser decreases soil pH (Liu *et al.* 2012). Long term application of inorganic fertilizer might be the factor leading to soil stress and acidification in oil palm plantation. Application of mineral fertiliser, in addition with high rainfall, led to leaching, pollution and wastage of fertilizers (Comte *et al.* 2013). Classification of soil nutrient status (Goh and K.J. 1997) showed that CEC 6 cmol kg-1 and total N lower than 0.08 % were very low for oil palm plantation. It proved this research when soil acidicity increased, i.e. pH decreased and more H^+ attached to the soil leading to decreased of available CEC. The CEC is an indicator of potential nutrient adsorption in organic mineral complexes, particularly the retention of basic cations (Zech *et al.* 1997). From result previously reported as support data continuous cropping often resulted in a decline in soil organic matter, which may be attributed to

the following factors, i.e. reduction on organic material, macronutrient and increased ion concentration in acidic condition. Macronutrient elements such as nitrogen, phosphorus, potassium, calcium and magnesium decreased with only aluminium and ferum increased. Micronutrient elements for boron, manganese, zinc and mangan decreased but silica increased. The present results revealed decline of pH, even though it decreased only 0.1 but increased ion aluminium, ferum and silica as indicator soil in acidic condition and decreased of some elements, such as nitrogen, total available phosphorus, calcium and magnesium indicating the soil is in stressed condition. Low pH, high Al activity in the soil solution, as well as Ca and Mg deficiency are major factors in reducing the yield of the crops in these soils (Shamshuddin and Fauziah, 2010). The result showed that long-term fertilizer application, especially of N, had acidifying effects causing a decrease in pH (Figure 4.4). This confirms earlier findings that most N containing fertilisers tend to acidify the soil (Belay *et al.* 2002). The high level variations in organic content, total nitrogen, available phosphorus, calcium, magnesium and potassium observed could be attributed to oil palm impact on the soil (Ogeh, and Osiomwan, 2012). Soil acidity can cause limited availability of some macronutrients and micronutrients such as phosphorus which binds to iron and aluminium oxides in acidic soils. On soil particle size distribution, there is a big difference in secondary forest soil and after 25 years application inorganic fertilizer on percentage of slit, coarse sand and fine sand. Increased percentage of clay and decreased percentage of slit, coarse sand and fine sand. Observation might be a general application management on oil palm plantation including usage a machine for harvesting and cutting grass. Data on soil properties and composition are strong data

that can proof that application of long term inorganic fertilizer can affect the soil. In this study, microbial profile using DGGE and MiSeq was used to observe on the effect of long term application inorganic fertilizer on soil microbes. As already reported, soil microbial community is critical to the maintenance of soil health and quality (Garbeva *et al.* 2006). The pH affects microbial activity, which in turn can affect the bioavailability of both macronutrients and micronutrients. Microbes can increase nutrient bioavailability and promote plant nutrient uptake; vegetable crops can also thrive in similar environments. In addition to the differences of microbial populations, the DGGE profiles show significant dissimilarities in the compositions of microbial communities in the different soil samples. Microbial activity is a term used to indicate the vast range of activities carried out by microorganisms in soil, whereas biological activity reflects not only microbial activities but also the activities of other organisms in the soil. Molecular fingerprinting techniques, including PCR-DGGE analysis, have become popular for assessing diversity, structural composition and dynamics of microbial communities (Nocker *et al.* 2007). Although PCR-DGGE allows the rapid assessment of the whole microbial community structure and identification of the dominant species, however, the analytical technique has some limitations: recovering DNA sequences information from excised gel bands ultimately requires cloning. Only relatively small fragment size of PCR products can be separated (up to 500 bp) and due to the limited success of direct sequencing from an electrophoresed gel, cloning of amplified 16S rRNA genes was conducted. Figure 4.6 uesd MiSeq after quality filtering Q20 on secondary forest soil compared with after 25 years application of inorganic fertilizer in soil, decreased in *Firmicutes* with *Bacteroidetes,* and

Acidobacteria and *Proteobacteria* increased between four top abundant phyla. The relative abundance of *Bacteroidetes* decreased caused by long term application inorganic fertilizer direct into soil which agreed with previous observation (Cui *et al.* 2018). This work continued the result with previous study that found that *Bacteroidetes* was very important as an indicator of soil health in vanilla monoculture system and black pepper on soil bacterial communities (Xiang *et al.* 2015). Moreover, the relative abundance of *Firmicutes* corresponded with soil borne diseases suppression. Phyla *Chloroflexi* as an indicator for organic carbon significantly with result on soil chemical composition decreases after 25 years application inorganic fertilizer. In Figure 4.5, the bacterial abundance significantly decreased with increasing years of oil palm plantation, bacterial diversity significantly decreased after 25 years oil palm plantation. Fierer *et al.* (2007) found that the soil microbial diversity had been almost completely eradicated after decades of intensely agricultural practices in tall grass prairies of United States. Soil microbial abundance and diversity have an important role in soil quality, functions and soil ecosystems sustainability. Hence, the loss of soil microbial abundance and diversity might be contributed to the crop poor dissimilarity, hence the loss of soil microbial abundance and diversity might be contributed to the crop poor growth in continuous cropping systems. In Figure 4.5 shows the result on microbes between fertilizer area and unfertilizer area with the same pattern, with *Acidobacteria* higher followed by *Acinobacteria, Proteobacteria, Firmicute, Verrucomicrobia,* and *Chlorofexi.*

Table 4.1 Soil analysis on macronutrient analysis between one cycle of oil palm plantation compare secondary forest

Plot	Area	Depth (cm)	pH (1:2.5)	Total N %	Org C %	CEC %	P (mg/kg) Total	P (mg/kg) Avail	K	Ca Exch cmol(+)/kg	Mg Exch cmol(+)/kg	Al Exch cmol(+)/kg
P1	FA	0-15	4.48±0.01b	0.06±0.03ab	1.11±0.24abc	5.99±0.40a	217.63±59.90a	5.72±0.40ab	0.11±0.01b	1.12±0.13ab	0.31±0.08a	0.23±0.06a
	FA	15-30	4.48±0.05b	0.07±0.03ab	0.87±0.40bc	5.43±1.04ab	179.38±21.10a	5.83±1.04a	0.12±0.04b	1.00±0.11ab	0.27±0.07a	0.30±0.11a
	UFA	0-15	4.46±0.05b	0.06±0.02ab	0.71±0.17bc	5.76±0.24a	154.13±42.80a	5.72±0.24ab	0.11b±0.01b	1.00±0.18ab	0.21±0.12a	0.28±0.01a
	UFA	15-30	4.46±0.05b	0.06±0.02ab	0.92±0.32bc	5.91±0.31ab	189.88±49.43a	5.83±0.31a	0.12±0.01b	1.10± 0.18ab	0.30±0.13a	0.31±0.05a
P2	FA	0-15	4.54±0.01ab	0.04±0.02ab	0.64±0.25c	5.07±0.66a	85.13±43.11a	5.72±0.66ab	0.11±0.04b	1.14±0.04ab	0.33±0.07a	0.47±0.06a
	FA	15-30	4.54±0.01ab	0.05±0.02ab	0.62±0.21c	5.14±0.50ab	85.13±41.48a	5.83±0.50a	0.12±0.02b	1.10±0.06ab	0.30±0.06a	0.47±0.03a
	UFA	0-15	4.51±0.05b	0.04±0.02ab	1.15±0.75bc	5.61±0.67a	153.88±78.37a	5.72±067ab	0.11±0.03b	1.22±0.40ab	0.55±0.34a	0.47±0.07a
	UFA	15-30	4.51±0.05b	0.04±0.02ab	0.84±0.42bc	5.25±0.65ab	117.13±85.95a	5.83±0.65a	0.12±.02b	1.44±0.12b	0.39±0.11a	0.57±0.07a
P3	FA	0-15	4.48±0.01b	0.03±0.02b	1.13±0.37c	4.80±0.82a	137.13 ±22.84a	5.72±0.83ab	0.11±0.03b	1.20±0.11ab	0.40±0.17a	0.39±0.05a
	FA	15-30	4.48±0.01b	0.02±0.01b	0.67±0.28c	4.70±0.28ab	81.38±49.86a	5.83±0.28a	0.12±0.05b	1.00± 0.09ab	0.27±0.08a	0.44±0.07a
	UFA	0-15	4.46±0.01b	0.05±0.03ab	1.19±0.27c	5.43±0.66a	128.13±77.09a	5.72±0.66ab	0.11±0.05b	1.20±0.22ab	0.40±0.16a	0.39±0.08a
	UFA	15-30	4.40±0.05b	0.04±0.02ab	0.90±0.35c	5.34±0.30ab	123.13±65.66a	5.83±0.30a	0.12±0.04b	1.20±0.19ab	0.32±0.15a	0.39±0.04a
P4	FA	0-15	4.43±0.06b	0.03±0.01a	1.07±0.54c	5.14±0.40a	141.88±41.98a	5.72±0.40ab	0.11±0.02b	1.20±0.20ab	0.38±0.18a	0.40±0.11a
	FA	15-30	4.41±0.05b	0.03±0.01b	0.63±0.25c	4.91±0.46ab	73.13±13.72a	5.83±0.46a	0.12±0.02b	1.00±0.12ab	0.24±0.11a	0.46±0.11a
	UFA	0-15	4.46±0.05b	0.06±0.04ab	0.95±0.05c	5.71±0.24a	117.88±37.26a	5.72±0.24ab	0.11±0.03b	1.20±0.17ab	0.31±0.03a	0.35±0.08a
	UFA	15-30	4.46±0.05b	0.02±0.02b	0.95±0.39c	5.31±0.49ab	107.13±42.59a	5.83±0.49a	0.12±0.03b	1.11±0.20ab	0.3±0.19a	0.39±0.10a
Soil Forest		0-15	4.70±0.10a	0.12±0.03a	2.30±0.44ab	3.57±0.87c	136.00 ±24.74a	3.13±0.50c	0.28±0.02a	1.00±0.20ab	0.23±0.04a	0.40±0.05a
		15-30	4.58±0.10ab	0.12±0.03a	2.74±0.74a	4.22±1.11c	157.75±60.57a	3.13±1.73c	0.28±0.02a	0.62±0.06b	0.21±0.04a	0.44±0.13a
Treatment			***	***	***	***	NSD	***	***	**	NSD	NSD
Depth			NSD	NSD	NSD	NSD	NSD	NSD	NSD	*	NSD	*
Treatment*Depth			NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD

Note: means with the same letter column are not significantly different at $p < 0.05$ according to Tukey (n=4)

Table 4.2 Soil analysis on micronutrient analysis between one cycle of oil palm plantation compare secondary forest

Plot	Area	Depth	B	Mn	Fe	Zn	Si	Mo	Particle-Size Distribution			
			mg/kg	mg/kg	mg/kg	mg/kg	mg/L	mg/L	Clay (%)	Slit (%)	Coarse sand (%)	Fine Sand (%)
P1	FA	0-15	1.83±0.23abc	182.28±11.26a	953.08±139.10a	1.96±0.07ab	11.55±54.78a	1.95±2.41a	48.42±0.79a	36.00±1.04ab	11.41±0.95abc	15.05±1.70b
	FA	15-30	1.27±0.40c	175.92±13.51a	789.42±227.84abc	1.93±0.13ab	122.92±25.28ab	1.31±2.61b	50.16±2.73a	36.35±2.21a	10.59±3.14ab	13.78±1.20b
	UFA	0-15	1.62±0.49abc	179.25±7.25a	814.94±59.21abc	1.92±0.02ab	96.31±22.26a	1.63±1.04b	48.46±0.95a	35.68±0.70ab	11.91±1.70abc	14.83±1.20b
	UFA	15-30	1.67±0.17abc	173.18±5.29a	856.63±105.18ab	1.95±0.05ab	102.97±16.21ab	2.50±1.44b	48.38±0.40a	35.23±0.26abc	12.04±1.64abc	15.25±1.37b
P2	FA	0-15	1.66±0.18abc	195.70±13.74a	655.64±103.21abc	1.65±0.03ab	55.46±6.83a	1.73±1.21b	48.58±2.94a	26.93±2.06bcd	11.61±2.44abc	12.40±2.11b
	FA	15-30	1.64±0.13abc	199.43±24.37a	647.55±97.12abc	1.61±0.12b	51.43±34.35ab	2.07±1.44b	47.55±3.13a	29.63±2.73bcd	12.74±2.85ab	12.43±3.63b
	UFA	0-15	2.00±0.45abc	180.85±28.11a	899.84±357.52a	1.76±0.16ab	93.68±33.71ab	2.29±2.00b	48.73±2.76a	29.33±1.10bcd	12.11±2.42abc	12.08±1.38b
	UFA	15-30	1.60±0.30bc	202.17±31.09a	615.95±179.10abc	1.59±0.12b	112.68±52.84ab	0.95±1.89b	47.45±3.56a	31.23±2.91abcd	12.04±2.79abc	11.33±1.44b
P3	FA	0-15	1.55±0.19bc	180.91±8.01a	844.73±144.54ab	1.79±0.07ab	67.37±2.76ab	1.20±1.97b	47.48±2.61a	21.40±3.06cd	13.34±4.27a	11.23±2.57b
	FA	15-30	1.31±0.20b	186.79±31.92a	618.47±124.84abc	1.69±0.08ab	89.37±55.18ab	2.46±1.99b	46.45±3.24a	21.48±3.76cd	14.84±2.77a	11.40±1.78b
	UFA	0-15	1.61±0.15bc	169.63±45.87a	821.21±69.08abc	1.76±0.20ab	58.91±32.32ab	1.01±1.26b	45.33±5.42a	20.00±4.86d	14.89±5.51a	13.25±3.18b
	UFA	15-30	1.45±0.14c	190.98±37.73a	725.10±89.79abc	1.75±0.20ab	90.56±25.54ab	1.90±2.11b	45.33±4.19a	21.20±5.07d	13.94±3.82a	12.08±3.23b
P4	FA	0-15	1.54±0.31bc	185.76±47.73a	782.10±224.38abc	1.85±0.16ab	56.18±39.44ab	3.18±2.67ab	46.18±2.40a	21.60±4.32cd	12.29±3.16abc	11.73±4.10b
	FA	15-30	1.50±0.30b	166.63±38.40ab	619.47±116.10abc	1.75±0.06ab	112.51±55.43ab	3.07±2.41b	48.20±2.00a	19.28±3.79d	12.29±2.97abc	13.68±1.98b
	UFA	0-15	1.65±0.17abc	185.11±24.84a	873.86±92.60a	1.82±0.05ab	97.66±64.05ab	1.84±2.21b	45.80±2.14a	20.00±2.61d	13.06±2.97a	14.28±0.67b
	UFA	15-30	1.22±0.25c	176.90±22.38a	797.43±120.80abc	1.79±0.05ab	98.66±36.27ab	1.30±1.53b	45.60±0.37a	20.00±1.36d	14.11±2.57a	10.85±7.34b
Soil Forest		0-15	2.43±0.26a	70.07±27.60bc	290.75±70.87c	1.84±0.18ab	22.35±4.25c	5.02±0.82a	43.50±0.91a	35.00±10.02abc	2.27±0.55c	28.23±2.38a
		15-30	2.43±0.24a	49.02±22.34c	332.08±55.51bc	2.10±0.26a	17.52±6.20c	5.83±1.64a	43.80±1.39a	41.02±6.62a	2.70±0.38bc	25.70±1.48a
Treatment			***	***	***	*	***	**	NSD	***	***	***
Depth			**	NSD	**	NSD	NSD	NSD	NSD	NSD	NSD	NSD
Treatment*Depth			NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD

Note: means with the same letter column are not significantly different at $p < 0.05$ according to Tukey (n=4)

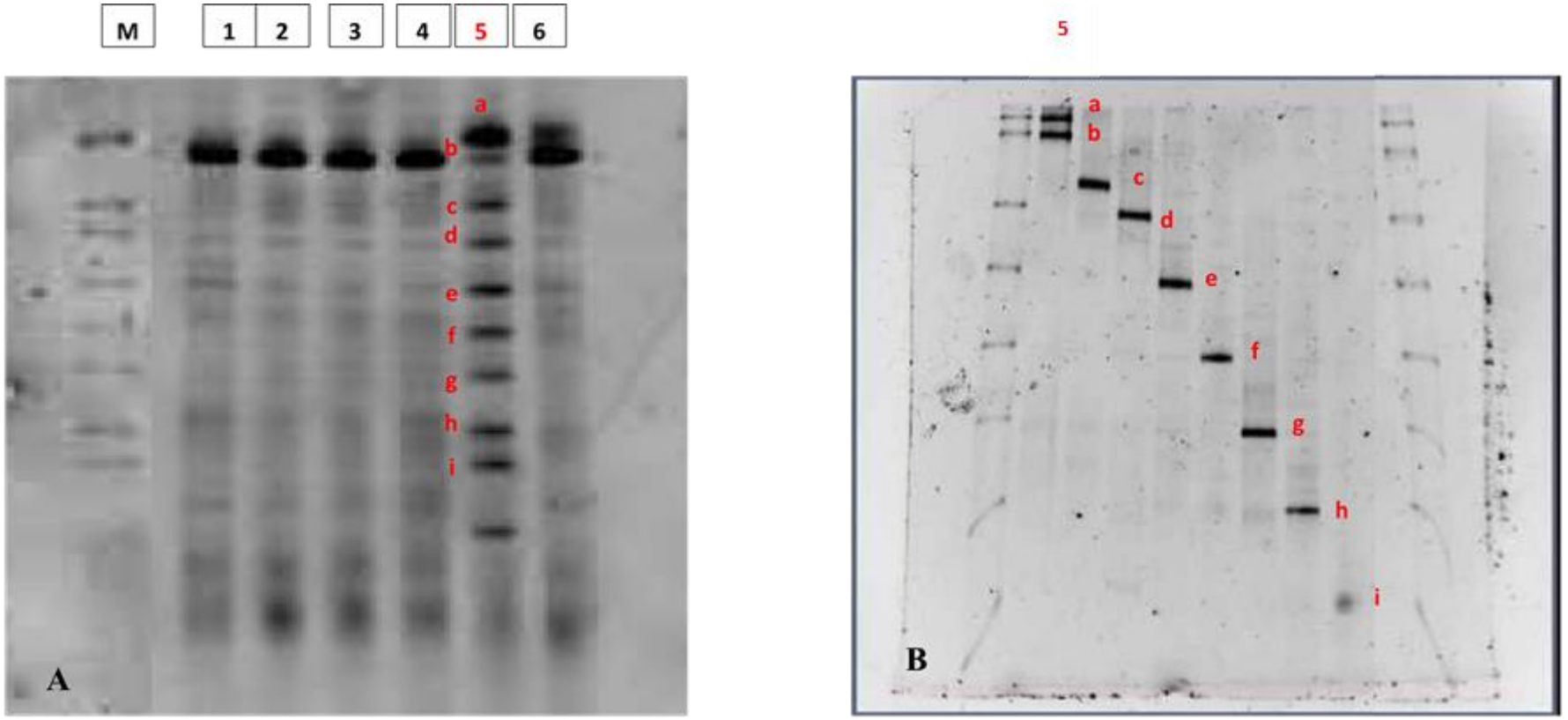


Figure 4.1 DGGE fingerprint profile first DGGE (A) and second DGGE on number 5-secondary forest 0-15 cm (B)
1. Fertilizer area; 0-15 cm, 2. Fertilizer area 15-30 cm, 3. Unfertilized area 0-15 cm,
4. Unfertilized area 15-30 cm 5. Secondary forest 0-15 cm, 6. Secondary forest 15-30 cm

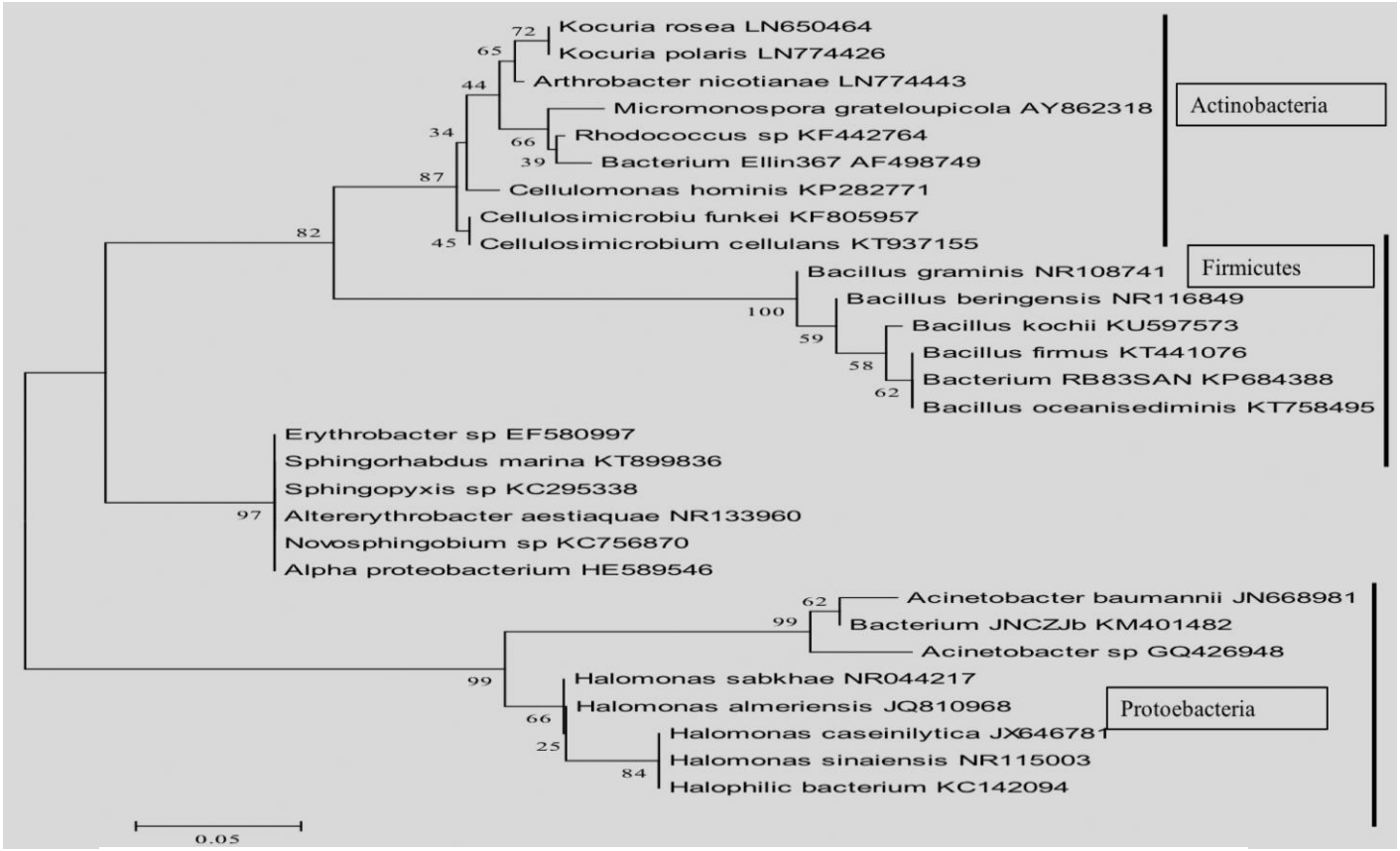


Figure 4.2 DGGE fingerprint on second DGGE for secondary forest 0-15 cm

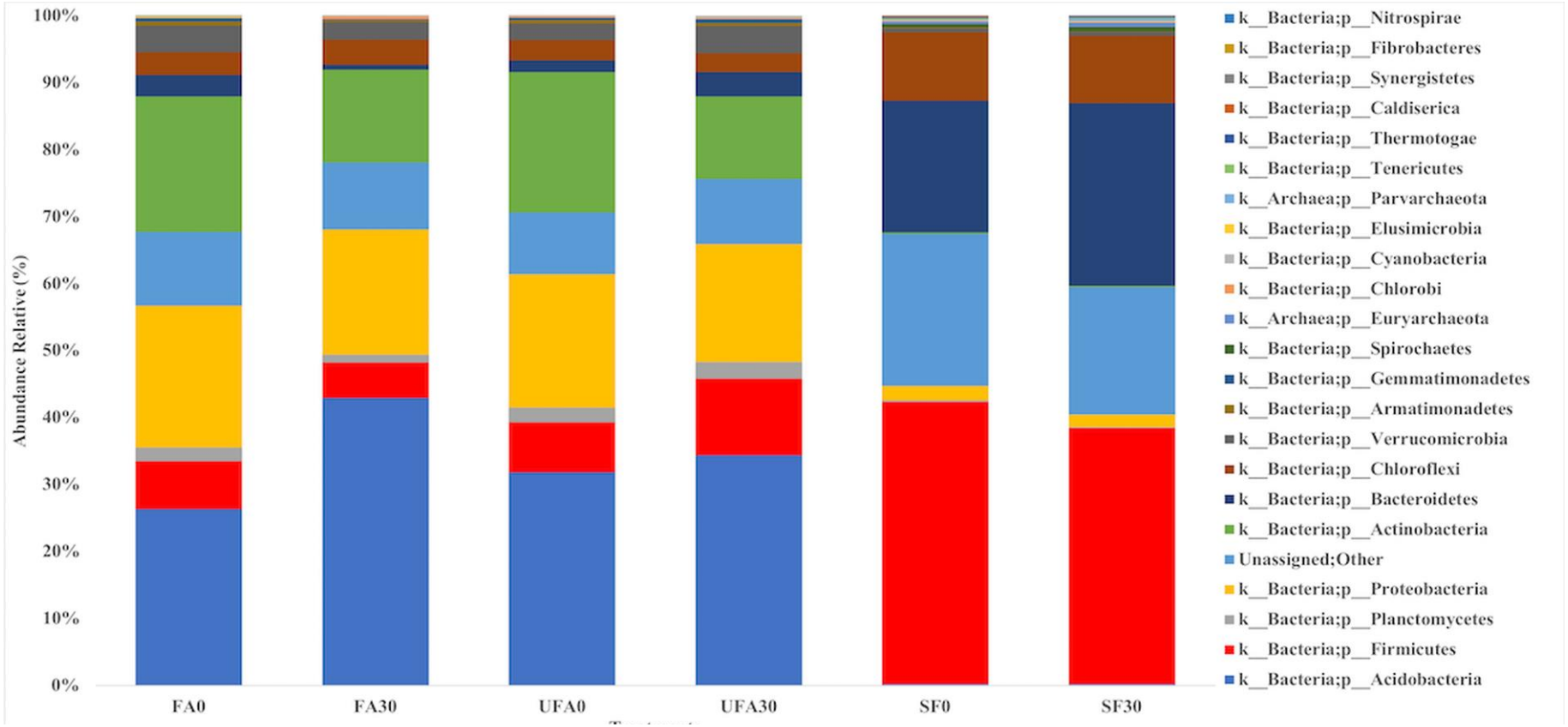


Figure 4.3 Relative abundance of the bacterial phyla (>1%) in the soil sampling
FA0 : Fertilizer area; 0-15 cm, FA30 : Fertilizer area 15-30 cm, UFA0 : Unfertilized area 0-15 cm, UFA30: Unfertilized area 15-30 cm, SFO : Secondary forest 0-15 cm, SF30 : Secondary forest 15-30 cm.

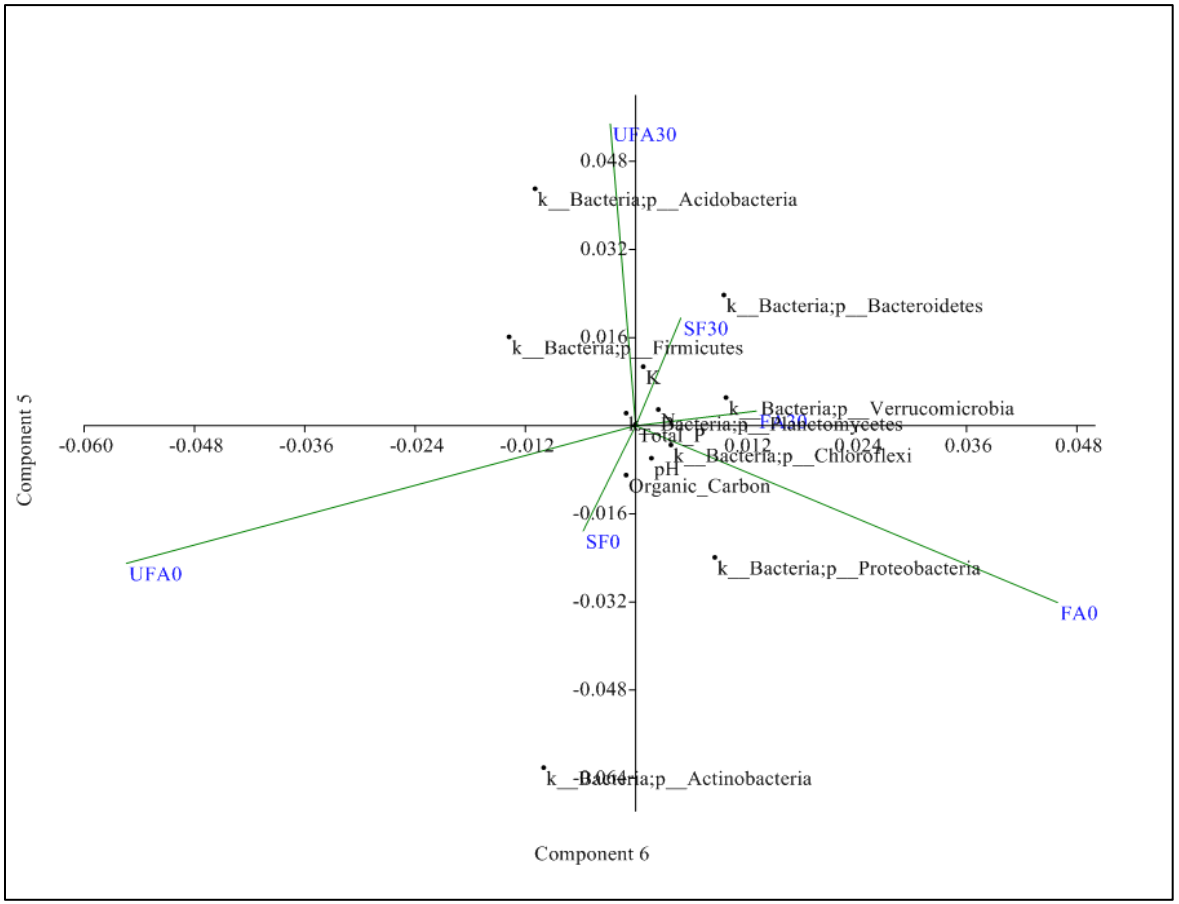


Figure 4.4 PCO ordinations of phylum based on relative abundance of OUT effect on treatments (FA0, FA30, UFA0, UFA30, SF0 and SF30). Variance explained in each axis is given parentheses.
FA0 : Fertilizer area; 0-15 cm, FA30 : Fertilizer area 15-30 cm, UFA0 : Unfertilized area 0-15 cm, UFA30: Unfertilized area 15-30 cm, SFO : Secondary forest 0-15 cm, SF30 : Secondary forest 15-30 cm.

Table 4.3 Percentage of relative abundance on genus of OTU effect on treatments

Kingdom	Phylum	Related Genus	Percentage of relative abundance					
			FA0	FA30	UFA0	UFA30	SF0	SF30
Archaea	*Euryarchaeota*	*Methanobacterium*	-	-	-	-	0.099	0.369
Archaea	*Euryarchaeota*	*Methanobrevibacter*	-	-	-	-	0.001	0.018
Archaea	*Euryarchaeota*	*Methanosphaera*	-	-	-	-	-	0.001
Archaea	*Euryarchaeota*	*Candidatus Methanoregula*	-	-	-	-	0.003	-
Archaea	*Euryarchaeota*	*Methanolinea*	-	-	-	-	0.006	0.003
Archaea	*Euryarchaeota*	*Methanospirillum*	-	-	-	-	0.009	0.01
Archaea	*Euryarchaeota*	*Methanosaeta*	-	-	-	-	0.164	0.096
Archaea	*Euryarchaeota*	*Methanomethylovorans*	-	-	-	-	-	0.006
Archaea	*Euryarchaeota*	*Methanosarcina*	-	-	-	-	0.001	0.003
Archaea	*Euryarchaeota*	*Methanomassiliicoccus*	-	-	-	-	0.004	0.017
Archaea	*Euryarchaeota*	*vadinCA11*	-	-	-	-	0.001	0.004
Bacteria	*Acidobacteria*	*Acidobacterium*	0.001	-	0.003	-	-	-
Bacteria	*Acidobacteria*	*Bryocella*	-	-	-	0.002	-	-
Bacteria	*Acidobacteria*	*Edaphobacter*	0.065	0.088	0.295	0.196	0.001	0.001
Bacteria	*Acidobacteria*	*Telmatobacter*	-	-	0.006	-	-	-
Bacteria	*Acidobacteria*	*Terriglobus*	0.009	0.007	0.031	0.035	-	-
Bacteria	*Acidobacteria*	*Candidatus Koribacter*	1.223	0.703	1.507	1.935	0.007	0.003
Bacteria	*Acidobacteria*	*Geothrix*	0.003	0.002	0.006	-	-	-
Bacteria	*Acidobacteria*	*Candidatus Solibacter*	2.511	2.529	3.629	2.664	0.016	0.025
Bacteria	*Actinobacteria*	*Actinotalea*	0.012	0.005	0.009	0.004	0	0.003
Bacteria	*Actinobacteria*	*Actinomyces*	0.103	0.035	0.057	0.028	0.001	-
Bacteria	*Actinobacteria*	*Actinoalloteichus*	0.001	-	-	0.002	-	-
Bacteria	*Actinobacteria*	*Actinokineospora*	0.001	-	0.003	-	-	-
Bacteria	*Actinobacteria*	*Arsenicicoccus*	0.001	0.005	-	0.002	-	-
Bacteria	*Actinobacteria*	*Actinomycetales*	0.001	-	-	-	-	-
Bacteria	*Actinobacteria*	*Actinocatenispora*	-	-	-	0.002	-	-
Bacteria	*Actinobacteria*	*Actinoplanes*	0.005	-	0.006	-	-	-
Bacteria	*Actinobacteria*	*Arthrobacter*	0.247	0.342	0.279	0.081	-	-
Bacteria	*Actinobacteria*	*Agromyces*	0.007	0.002	0.006	0.009	-	-
Bacteria	*Actinobacteria*	*Brevibacterium*	0.004	-	-	-	-	-
Bacteria	*Actinobacteria*	*Cellulomonas*	0.038	0.021	0.013	0.013	0.001	-
Bacteria	*Actinobacteria*	*Corynebacterium*	0.007	-	-	-	-	0.003
Bacteria	*Actinobacteria*	*Catellatospora*	0.001	-	-	0.002	-	-
Bacteria	*Actinobacteria*	*Clavibacter*	0.002	-	-	-	-	-
Bacteria	*Actinobacteria*	*Cryocola*	0.22	0.1	0.17	0.183	-	-
Bacteria	*Actinobacteria*	*Curtobacterium*	0.062	0.051	0.088	0.044	-	-
Bacteria	*Actinobacteria*	*Candidatus Microthrix*	0.001	-	-	0.002	-	-

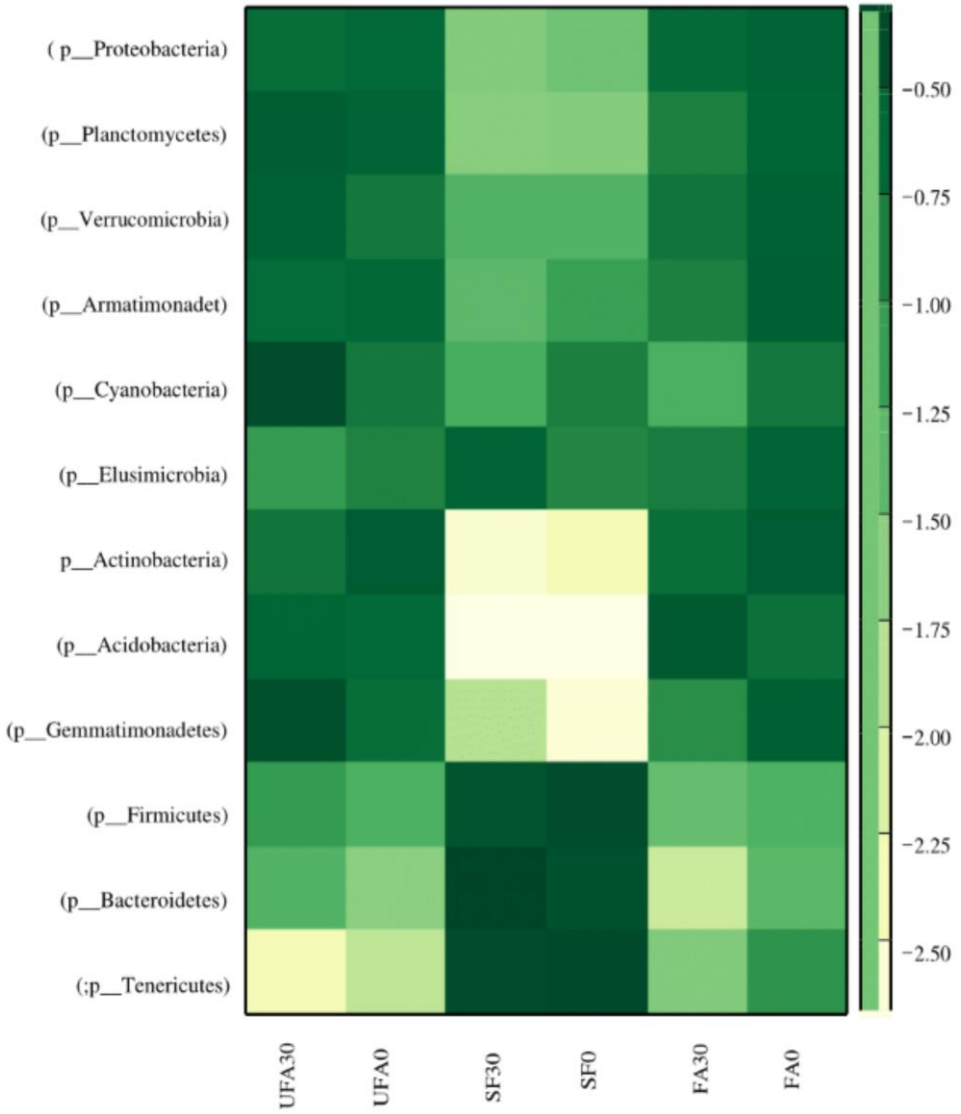


Figure 4.5 Relative abundance of the bacterial phyla (>1%) in the soil sampling.
FA0: Fertilizer area; 0-15 cm, FA30 : Fertilizer area 15-30 cm,
UFA0: Unfertilized area 0-15 cm, UFA30: Unfertilized area 15-30 cm,
SFO: Secondary forest 0-15 cm, SF30 : Secondary forest 15-30 cm.

4.4 Conclusion

Based on the result on nitrogen in soil after 25 years application of inorganic fertilizer in oil palm plantation, the value was very low from standard soil. Low cation exchange capacity and high aluminium, silica, zinc and ferum showed that the soil was in stress condition. Aluminium, ferum and zinc are indicators that the soil is under stress condition. The important finding for microbial diversity is that after 25 years application of inorganic fertilizer, *Firmicutes* which is important for soil borne diseases suppression decreased, as well as *Bacteriodete*s which is an indicator of soil health in forest soil. Fertilizer area and unfertilizer area shown the same pattern on microbial diversity. This result is interesting for investigating the long term effect of chemical fertilizer in for oil palm plantation. Our result suggests that to recover soil from this situation, application of organic fertilizer can one of the candidate solution. For our next research, we will use inorganic fertilizer and organic fertilizer on this plot for five years planting. We will also look whether application organic fertilizer will effect growth and yield of oil palm. This study is one of the few research on oil palm management that has taken place within oil palm blocks, embedded within a commercial production process. The research uses an industrial value for the country, is unique, with a comprehensive set of data on soil properties and microbial diversity compiled over four years, with industrials plots using consistent methods across space and time. While undertaking research within the inherently highly variable 'real world' context can make it more difficult to separate the influence of external environmental variables, commercial blocks makes our results highly relevant to those parties interested in improving oil palm management practices.

CHAPTER 5

EFFECT OF LONG TERM COMBINED ORGANIC AND INORGANIC FERTILIZER ON PLANT GROWTH, SOIL RESPONSE, MICROBIAL ACTIVITY AND OIL PALM PRODUCTION

5.1 Introduction

Malaysia is one of the world's largest producers of palm oil. The growth of oil palm plantation has posed environment problems in terms of deforestation, habitat destruction, greenhouse gas emission and soil and water quality depletion (Vijay *et al.* 2016). Increased production is achieved by increasing inorganic fertilizer input. Unfortunately, inorganic fertilizer has contributed to the impact of soil acidification, lowers pH and reduced the buffering ability of these tropical soils with low fertility. Commercial oil palm plantations which operate a processing mill have two sources of organic fertilizer available. One of them is POME anaerobic sludge from the mill containing organic carbon (including oil and fat), minerals, suspended solids and microorganisms. Research carried out since the 1980s has shown that POME and EFB can substitute mineral fertilizers to sustain oil palm yields and soil fertility by significantly increasing soil pH, water keeping ability, organic carbon quality, total nitrogen content, cation exchange capacity (CEC), usable phosphorus content and exchangeable non-acid cations (Abu Bakar *et al.* 2011). Such favourable effects are due to an increase in soil moisture, soil composition, organic matter quality and microbial growth, as well as fertilizer incorporation and a decrease in soil erosion and nutrient depletion (Trivedi *et al.* 2016).

In this objective, the effect of inorganic fertilizer application for second cycle of oil palm plantation with different combinations of compost is studied. Data for 5 years are collected and assessed on soil physiochemical, bacterial productivity, bacterial population dynamics, oil yield and economic statistics.

5.2 Materials and methods

5.2.1 Description of the site study

The soil samples were collected from the field site situated in enrichment planting (55º 40'S, 12º 18'E) (Figure 5.1). The study was conducted in Stesen Penyelidikan FASSB (2J) Serting Hilir 72120 Bandar Seri Jempol, Negeri Sembilan with daiy temperature variation of 24 – 33^{0}C. A plantation plot of 3.42 hectares was chosen. The study started from December 2013 and ended in December 2018, with plot size 5 x 6 palms (3 x 4 recording) using planting material PB 22 (DURA x GMH 43), type of soil Collovium and Durian, with terrains of flat, with RCBD seeding and plant distance 9.1 meters (30 plant). Planting density was 136 plants ha $^{-1}$ in 9.0 equilateral, triangular pattern. Plants were 1 year old at the onset of the study (December 2013) (Figure 5.2). The experiment took advantage of the replicated block design already in place (figure 5.3 and 5.4). Fertilization was managed according to age of oil palm cultivation. Oil palm seed, Dura x Pesifera, were obtained from FELDA Agriculture Services Sdn. Bhd., Pusat Penyelidikan Tun Razak, 26400 Bandar Jengka, Pahang Darul Makmur. Oil palm waste of pressed oil palm fruit mesocarp was taken from Jugra Palm Oil Mill Sdn Bhd, Lot 340 Jalan Tok Mujir Sungai Buaya Banting, 42700 Malaysia and POME sludge from Kilang Sawit Besout, FELDA Besout Besout Perak. Compost was produced from EFB and POME anaerobic sludge at pilot plant in Biomass and Biorefinery Laboratory

(BBL) Universiti Putra Malaysia, Serdang Selangor. The experimental plot was part of a larger, long term experiment covering about 3.24 ha. The growth of seedling were monitored at three, six and eight months after transplanting by recording the girth size, plant height, frond production, frond length and dry weight. The greenness of oil palm seedling or chlorophyll content was measured using SPAD 502 Chlorophyll meter.

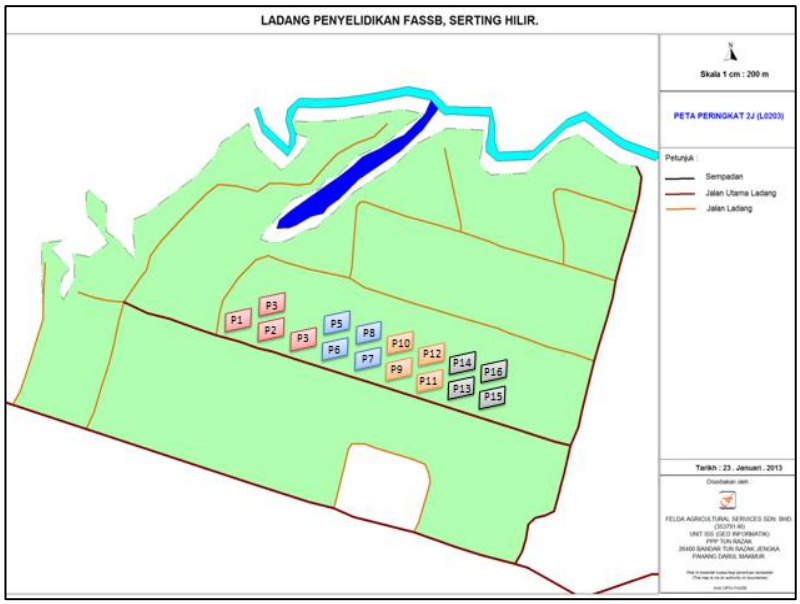


Figure 5.1 Part of an experimental used diamond shape

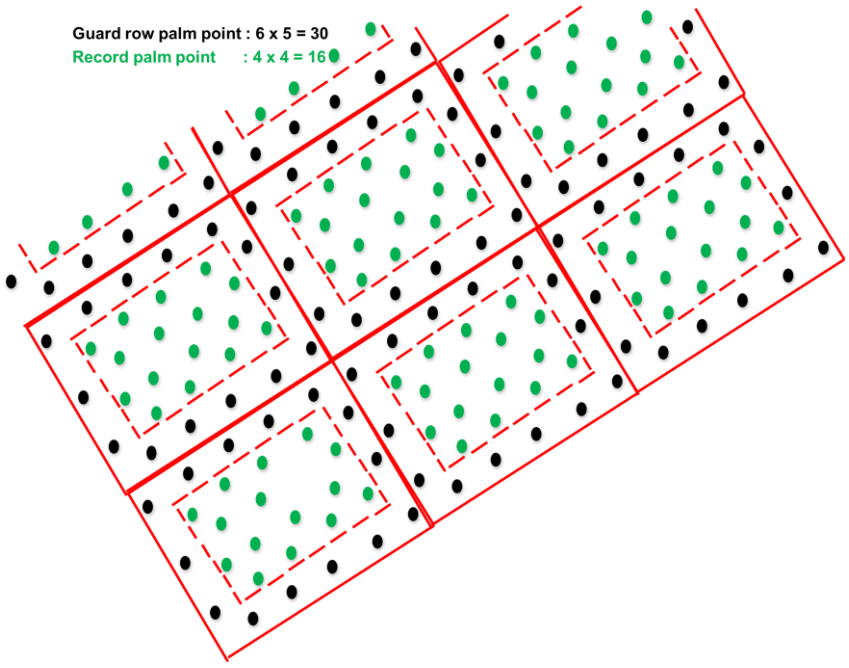


Figure 5.2 Sampling area at FELDA Serting Hilir Negeri Sembilan

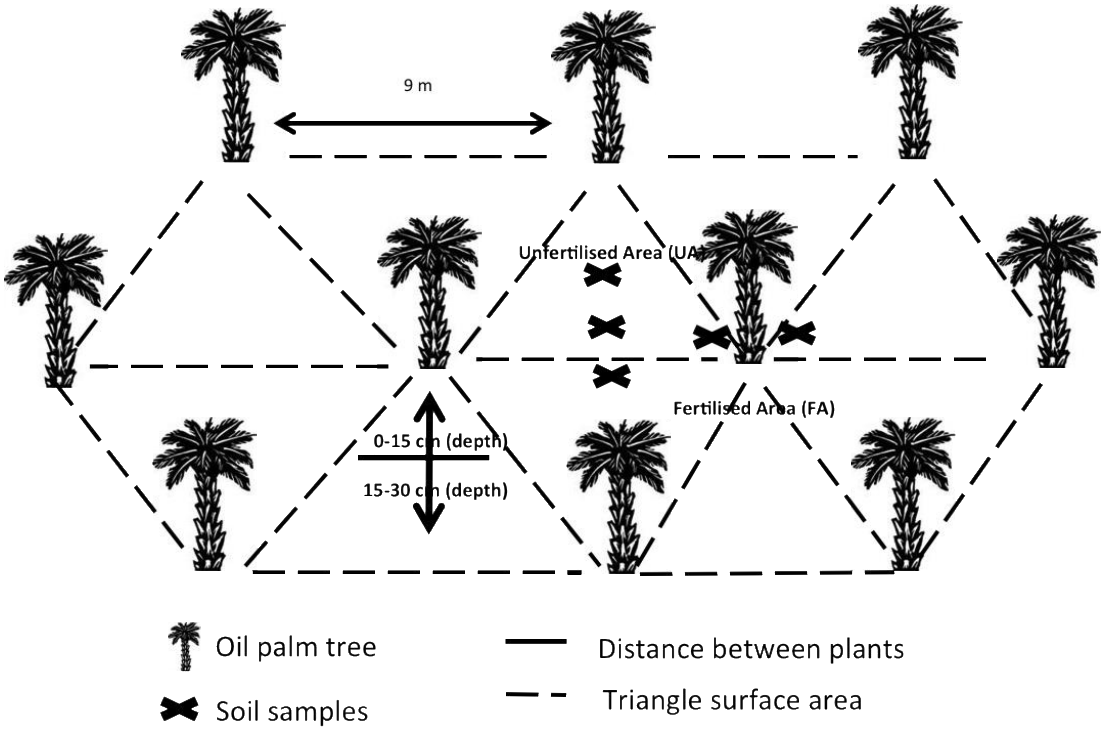


Figure 5.3 Layout sampling diagram between fertilizer area and unfertilized area

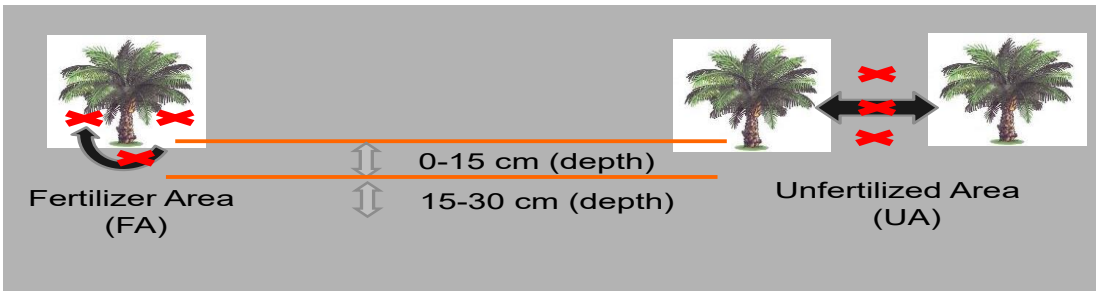


Figure 5.4 Layout sampling diagram between fertilizer area and unfertilized area

5.2.2 Organic fertilizer (compost)

5.2.2.1 Organic fertilizer technology

Composting technology has been introduced and used in some form since ancient times. A well-managed composting facility should exist as a good neighbour contributing to ecology. The new approach now is to reduce the duration taken from raw materials until stabilized/ matured compost. This can be done by enhancing the condition of anaerobic sludge and maintain it under controlled conditions.

5.2.2.2 Materials

The oil palm biomass are empty fruit bunch (EFB) and palm oil mill effluent (POME) anaerobic sludge.

5.2.2.3 Pome oil mill effluent (POME) anaerobic sludge

The POME anaerobic sludge was transported by lorry tanker from the nearby palm oil mill to the Compost Pilot Plant and loaded into the anaerobic sludge tank. The mixer paddle (impeller) will mixed the anaerobic sludge and make it suspended before mixing it with EFB. The tank is equipped with feed pump to transfer the anaerobic sludge to the windrow system as necessary.

5.2.2.4 Open windrow or bedding system

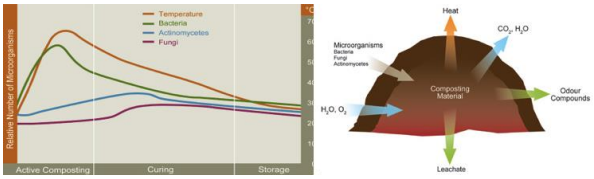
This method is suitable for the production of large amounts/volumes of compost. Piles are usually used to increase porosity and oxygen content, blend or extract head, and to redistribute cooler and hotter parts of the stock. Windrow composting is the most widely used method of farm scale composting. The process control parameters include the initial ratios of carbon and nitrogen-rich materials, the amount of bulking agents added to ensure air porosity, pile size, moisture content and frequency of turning. The height of the pile should be between 0.5 to 1.0 m with a base of 1.3 m to 1.5 m. The prepared mixture of anaerobic sludge need to be applied uniformly over the windrow piles. The initial temperature is not very critical to be controlled since the compost will create its own temperature due to the high microbial activity (Figure 5.5). A windrow turner machine was used to turn over the windrow pile uniformly (figure 5.6). It turned the compost pile heap inside-out and distributed the mixture evenly (Baharuddin *et al.* 2010).

Figure 5.5 Environmental friendly feature of compost process at Biomass Technology Laboratory (BTL) Universiti Putra Malaysia, Serdang Selangor.

Uniform used for processed compost

POME Sludge putted into EFB

POME Sludge mix with EFB using turning machine



Theoretical temperature variations and microbial populations during the composting process & the composting process

Figure 5.6 Theoretical and application of compost process at Biomass Technology Laboratory (BTL) Universiti Putra Malaysia, Serdang Selangor

5.2.3. Oil palm plantation approach

Oil palm plantation approach was developed to assess the soil response to long term organic fertilizer and mineral fertilizer application. This approach is based on analysis of soil responses when there are no historical records of soil analysis but only historical fertilizer application. It is assumed that the initial soil fertility level was the same for a given plantation. An approach to oil palm planting was established to determine the reaction of soil to long-term application of organic fertilizer and mineral fertilizer. It is assumed that for a given plantation the initial soil fertility level was the same.

5.2.3.1 Inorganic fertilizer application

The historical record of inorganic fertilizer and organic fertilizer application for each block was obtained from the plantation manager, which included data from a 5 years period, from 2014 to 2018 inclusive. Some blocks had a uniform fertilizer sequence (100% inorganic fertilizer: 0% organic fertilizer, 50 % inorganic fertilizer: 50 % organic fertilizer, 25 % inorganic fertilizer: 75 % organic fertilizer and 0 % inorganic fertilizer: 100 % organic fertilizer) during the study period, but most blocks had mixed fertilizer sequences. Given the variability among mixed fertilizer sequences in this plantation, blocks that received similar mixed fertilizer sequences were grouped before comparative statistical tests was done.

5.2.4 Measurement of vegetative growth in immature palms

Figure 5.7 shows sampling for plant growth: rate of leaf production, total number of leaves, petiole cross section, trunk diameter and rachis length.

5.2.4.1 Rate of leaf production

Frond 1 which is the youngest fully opened frond at the start of the recording period was marked 1. The most conspicuous colour to use is light blue. Left handed palms should be counted right from frond 1 and right handed palms to the leaf. Estimate the number of new leaves produced during period if the previously marked frond 1 is now the third frond from the top in parastichy 4 next to parastichy 1, 19 new leaves have been produced. If the recording period was 12 months, frond production rate 19/12 =1.6 frond months^{-1}. Mark a new frond 1 at least one per year or at every survey to provide a new refence point before the oil frond 1 is pruned off. Depending on palm age and environment, the rate of leaf production should be 18-40 leaves year^{-1} or 1.5-3.3 fronds month^{-1} (Tiong, 1999).

5.2.4.2 Total number of green leaves

Remove sufficient old leaf bases, at about 1.5 m above ground level (above the basal bulge), to expose about 5 cm^2 of trunk at two on opposite sides of the trunk. Trunk diameter ranges from 0.30 to 0.50 m but is greater at basal bulge (Tiong, 1999).

5.2.4.3 Petiole cross section

Select either a standard leaf (frond 17) or measure those leaves marked as frond 1 for leaf production records. Measure width in cm of petiole at point of insertion of the lowest rudimentary leaflet. Measure depth at the same point with callipers or cut

through the petiole at the same point, and measure the depth across the cut with a ruler. Petiole cross section (PCS) meaning width times depth. The PCS value increases with palm age, up to about 60 cm^2. Petiole cross section is highly correlated with leaf dry weight, total palm dry weight and vegetative dry matter production (Tiong, 1999).

5.2.4.4 Trunk diameter

Remove sufficient old leaf bases, at about 1.5 m above ground level (above the basal bulge), to expose about 5 cm^2 of trunk at two on opposite sides of the trunk. Trunk diameter ranges from 0.30 to 0.50 m but is greater at basal bulge (Tiong, 1999).

5.2.4.5 Rachis length

Select a standard leaf at frond 17 or measure those leaves marked as frond 1 for leaf production records. Measure length from the point of insertion of lowest rudimentary leaflet to the tip of rachis.

A. Rachis x-Section

B. Width of Leaves

C. Sampling Leaves

D. Number of Leaves

E. Canopy Length

Figure 5.7 Sampling for plant growth of oil palm plantation at FELDA Serting Hilir Negeri Sembilan.

5.2.5 Soil fertility survey and soil analysis

The next step was to perform a one-off soil survey at the landscape scale to assess the current soil fertility status across the plantation. Soil survey was done every December from 2014, 2015, 2016, 2017 and 2018 in the 3.2 hectares plantation. We selected 192 plants of the 16 plots within the plantation, and within those blocks collected soil samples at a density of one sampling location per two hectares for assessment of soil fertility status. Due to the heterogeneous structure of the oil palm plantation, a stratified soil sampling method was employed to account for intra-block variability (Maena *et al.* 1979; Law *et al.* 2009). This involved taking three sub-samples of soil (0–15cm depth) from three zones in the vicinity of a palm tree: the palm circle, the harvest path and the frond piles. All sub-samples from a particular zone (*e.g.*, palm circle) were mixed to obtain a representative sample from that block, and then composited with samples from the same zone, taken from other locations in the block. In total, 288 composite soil samples (96 blocks × 3 zones) were collected. Due to the heterogeneous structure of the oil palm plantation, a stratified soil sampling method was employed to account for intra-block variability (Maena *et al.* 1979; Law *et al.* 2009). This involved taking three sub-samples of soil (0–15cm depth) from three zones in the vicinity of a palm tree: the palm circle, the harvest path and the frond piles. All sub-samples from a particular zone (*e.g.*, palm circle) were mixed to obtain a representative sample from that block, and then composited with samples from the same zone, taken from other locations in the block. In total, 288 composite soil samples (96 blocks × 3 zones) were collected. Weigh 20 g of soil (2 mm) into a plastic bottle. Distilled water was added into the bottle. The soil was shaken for one hour of intermittent shaking and overnight standing. pH was calibrated by buffer pH

4.00 and pH 7.00. Basic exchangeable cations were extracted determined by electrolyte solution in 0.01 M KCI. CEC was determined with ammonium acetate and determining of ammonium ions by the soil used colorimetric method. The total organic C content of the soils was determined using Walkely and Black titration method (Gelman *et al.* 2012). The total N content of the soil was determined using Alkaline Phenol and Hypochlorite. To measure P content, soil sample first digest for 1 ¾ hours using Block Digester at 200º C (high temperature) and analysis of P using the Auto-Analyser 1(Amin *et al.* 2004). Determinate of Soil Exchangeable cation (K, Ca, Mg, Na) by 1M Ammonium Acetate. The potassium, magnesium and calcium determination on AAS pipette 2 ml of the original solution and add 20 ml of 825 ppm Strontium Nitrate by using Auto-Diluter 111. The sodium determination, use the original solution and read on AAS. Analytical results represent the nutrient levels and other soil physicochemical parameters in mineral soil, which did not include undecomposed residues (EFB, fragments of vegetation and other organic residues) since those residues were not included at the time of sampling and visible fragments were removed prior to analysis.

5.2.6 Harvesting procedure

Figure 5.8 shown fruit fresh bunch (FFB) of oil palm. Harvesting and recording of FFB yield was carried out for each whole block by the collaborating plantations. This was done for five years at each of the six sites by the estates where the project blocks were located. Recording was done as in the normal way that estates operate:

1. All harvested bunches brought to the roadside platforms were counted to obtain the total bunch number for each block
2. These bunches were then transported to the nearest oil palm mill
3. Weighed at the mill weighbridge to obtain total weight of FFB for each block.

5.2.6.1 Bunch analysis procedure

Figure 5.9 shows analysis of oil extraction oil palm bunch from field FELDA Serting Hilir Negeri Sembilan until processed at Central Laboratory Tun Razak Sungai Tekam Pahang. Bunch analysis (BA) was implemented during the final year of the BMP projects to estimate oil and kernel contents of the FFB from each whole block, so that the recorded FFB yields could be expressed in terms of oil and kernels. At each site, the required BA laboratory (BA Lab) facilities were established by the collaborating plantations, and a team was formed and trained to undertake the BA procedure. BA is a procedure in which the components of a single bunch are determined in step-wise samplings, starting from a whole bunch until oil is extracted from a small sample of dried mesocarp. BA is primarily used by oil palm breeders to determine oil and kernel yield potential and other highly heritable fruit traits of individual palms in breeding programmes; it is used sparingly in agronomy research. In the BMP projects, the BA procedure was adapted for the estimation of oil and kernel content of whole blocks. For each block, the ratios of fruits-per-bunch (F/B), wet mesocarp-per-fruit (M/F), oil-per-mesocarp (O/M), and kernel-per-fruit (K/F) of the harvested FFB were determined (Blaak *et al.* 1963).

From these parameters, the O/B ratio (i.e. oil content in FFB) for the block was calculated from the following equation:

O/B = F/B x M/F x O/M

The O/B value from BA was corrected by a factor of 0.855 to give the potential mill OER.

Similarly, the kernel per bunch (K/B) ratio (i.e. kernel content in FFB) for the block was obtained by the following equation:

K/B = F/B x K/F.

The oil and kernel contents determined through BA was done on bunches that did not undergo the normal pre-extraction conditioning of an oil palm mill (i.e. high pressure steam sterilization). And solvent extraction was used to determine oil content of the mesocarp, as opposed to the physical extraction process used in palm oil mills. (Blaak *et al.* 1963)

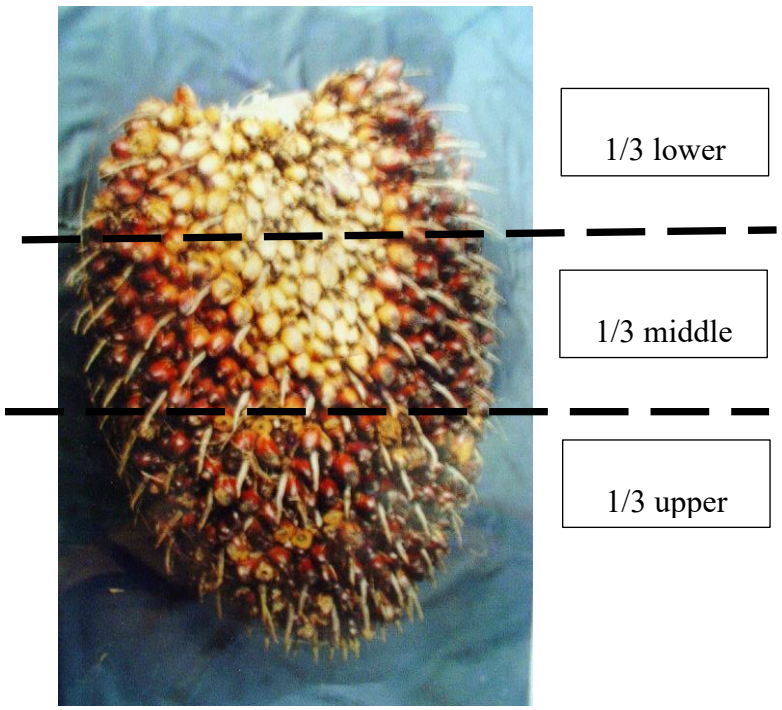


Figure 5.8 Fresh fruit bunch (FFB) according to distribution of oil palm bunch

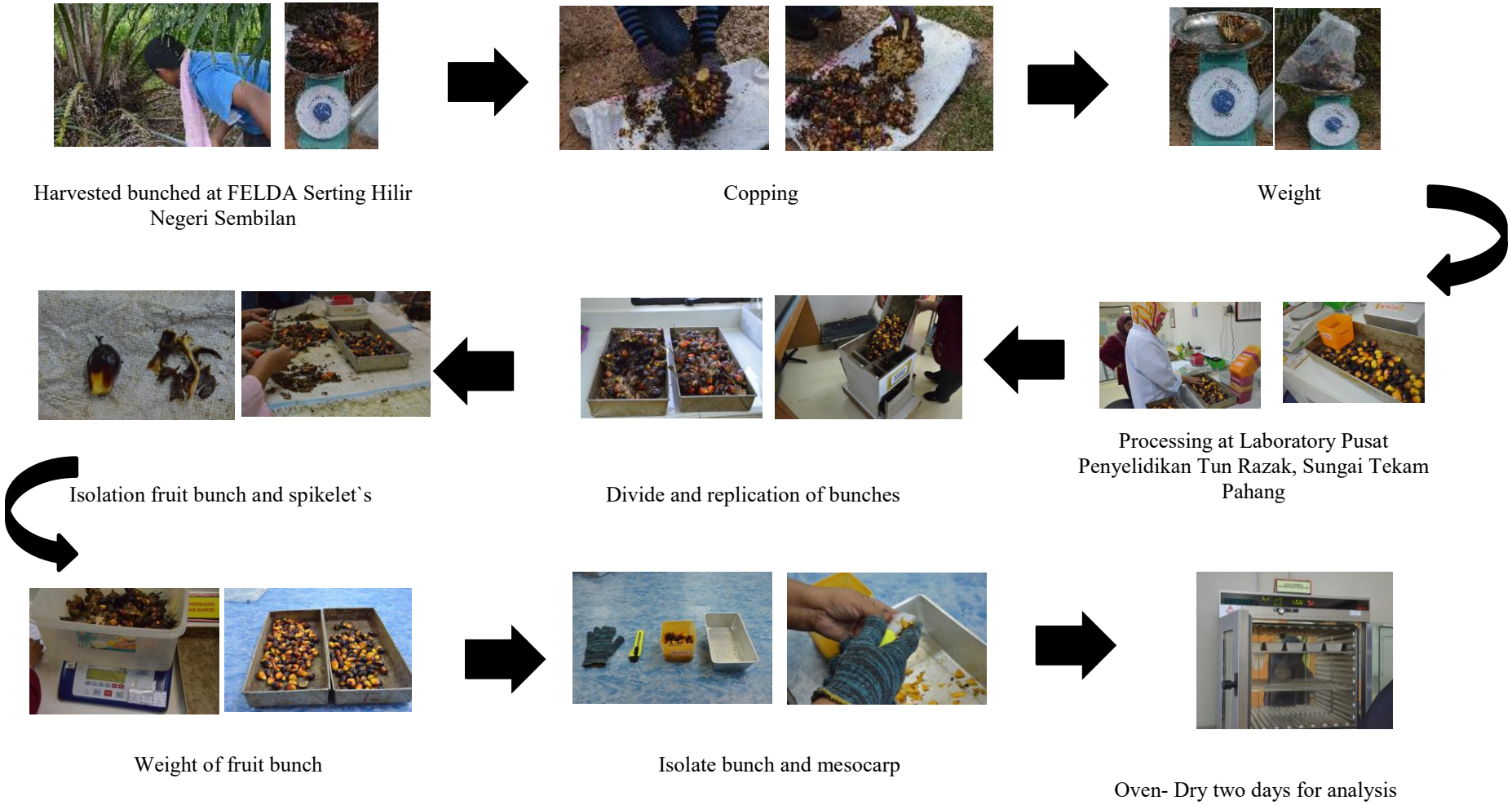


Figure 5.9 Analysis of oil extraction oil palm bunch from field FELDA Serting Hilir Negeri Sembilan until processed at Center Laboratory Tun Razak Sungai Tekam Pahang.

5.2.7 High-throughput 16S rRNA sequencing

The high-thoughtput 16S rRNA sequencing method described in **Section 3.3.**

5.2.8 Statistical analysis

Data for soil properties and nutrient depletion were identified by analysis of variance (ANOVA) using the SAS Software Windows Version 8 (SAS, 2001). Turkey analysis at $p \leq 0.05$ was used to test significant difference between the treatments. Tukey's honestly significantly different test for all pairwise comparisons were calculated after ANOVA to compare treatment means. Statistical analysis of DGGE profiles, using Gel images of the DGGE profiles were converted, normalized and digitized using Quantity One 3.0software (Bio-Rad). QIIME analysis used versions V.9.0, to perform OTU clustering and alpha and beta diversity analyses. Reference-based OTU clustering was done using the parallel uclust_ref method while *de novo* OTU clustering was done with standard uclust, using the default options as implemented in QIIME for both methods at the 97% similarity level. Coverage, richness (Chaol and ACE indexes), and diversity Shannon indices were used to estimate the alpha diversity.

5.3 Results and discussion

5.3.1 Organic fertilizer (compost) characteristic

POME anaerobic sludge and EFB were substrates for the organic fertilizer. The POME anaerobic sludge is a colloidal suspension discharged from the factory into open wastewater treatment for anaerobic digestion followed by aerobic treatments (Baharuddin *et al.* 2009). Table 5.1 showed organic fertilizer as compost on pH (8.05), organic matter (80.5 %), total carbon (44.85 %), total nitrogen (3.16 %), carbon and nitrogen ratio (14.5 %), phosphorus (0.65 %), potassium (3.98 %), calcium (1.30 %), and magnesium (0.57 %). Figure 5.11 shows the use of irrigation tube once (750 m^3 ha^{-1}) through the bedding scheme and POME anaerobic sludge. For the composting process to complete without the addition of inoculants took about 40-45 days. The composition and diversity of microbial community at various phases of the co-composting process was adequately evaluated by giving an in-depth assessment of the 16S rRNA gene MiSeq. The development of bacterial diversity as co-composting progressed due to the action by microbes in the POME anaerobic sludge on the easily decomposable organic products, backed by the decreased of the complete carbon and C/N ratio throughout the co-composting phase in this research. Figure 5.10 shows comparative compost as an organic fertilizer in phylum between in year 1 (2014) at the initial growth of oil palm and after five years growth of oil palm plantation. *Bacteroidetes, Actinobacteria, Gemmatimonadetes, Chloroflexi, Plantoycetes, Firmicutes, Verrucomicbia, Acidobacteria, Termi* and *TM7* follow relative abundance wealthy in *Proteobacteria.* Related in genus, 2014 (554) and 2017 (607) elevated *Inquilinus*. Table 5.2 demonstrates phylum dominant *Proteobacteria* associated family with *Rhodospirillacease* and the genus with *Paracoccus.* Table 5.3 demonstrates

Actinobacteria, the *Glycomycetacease* family associated with *Glycomyces* genus. Related *Sphingobacteriacease* in *Parapedobacteria* for *Bacteriodetes*. Continue with *Firmicutes*, family *Bacillacease,* and genus *Bacillus.*

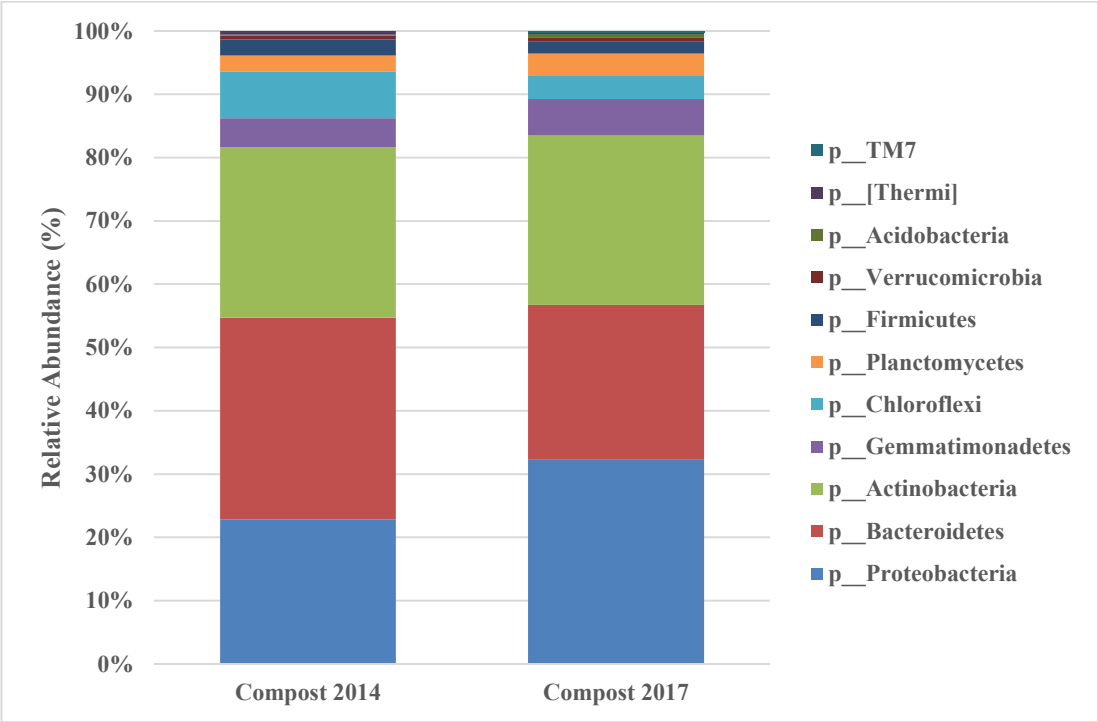


Figure 5.10 Relative Abundance of phylum inorganic fertilizer (compost) at 2014 and 2017(>1%)

Table 5.1 Nutrient content of inorganic fertilizer (compost)

Inorganic Fertilizer	pH	Organic Matter (%)	Total Carbon (%)	Total Nitrogen (%)	C/N Ratio	P (%)	K (%)	Ca (%)	Mg (%)
Compost	8.05	80.5	44.84	3.16	14.5	0.6	3.98	1.3	0.57

Figure 5.11 Production of inorganic fertilizer (compost) 2014, 2015, 2016, 2017 and 2018 at Biomass Technology Laboratory (BTL) Universiti Putra Malaysia, Serdang

		
2014 – 6 tans	**2015 – 12 tans**	**2016, 2017 & 2018 - 20 tans**

Table 5.2 Percentage of relative abundance in organic fertilizer (compost) on *Proteobacteria* for 2014 and 2017

Phylum	Related Family	Related Genus	Percentage of relative abundance	
			Compost 2014	Compost 2017
Proteobacteria	Rhodospirillaceae	Inquilinus	21	12
Proteobacteria	Rhodospirillaceae	Paracoccus	6	13
Proteobacteria	Xanthomonadaceae	Luteimonas	-	14
Proteobacteria	Phyllobacteriaceae	Mesorhizobium	-	2
Proteobacteria	Sinobacteraceae	Steroidobacter	4	1
Proteobacteria	Hyphomicrobiaceae	Pedomicrobium	2	-
Proteobacteria	Alteromonadaceae	Cellvibrio	-	1
Proteobacteria	Nannocystaceae	Plesiocystis	-	1
Proteobacteria	Alcaligenaceae	Bordetella	-	1
Proteobacteria	Xanthomonadaceae	Pseudoxanthomonas	-	1
Proteobacteria	Hyphomicrobiaceae	Rhodoplanes	-	-
Proteobacteria	Halomonadaceae	Halomonas	-	-
Proteobacteria	Beijerinckiaceae	Chelatococcus	-	-
Proteobacteria	Moraxellaceae	Acinetobacter	-	-
Proteobacteria	Piscirickettsiaceae	Methylophaga	-	-
Proteobacteria	Rhizobiaceae	Agrobacterium	-	-
Proteobacteria	Alcaligenaceae	Alcaligenes	-	-
Proteobacteria	Xanthomonadaceae	Pseudofulvimonas	-	-
Proteobacteria	Alcanivoracaceae	Alcanivorax	-	-
Proteobacteria	Hyphomicrobiaceae	Hyphomicrobium	-	-
Proteobacteria	Pseudomonadaceae	Pseudomonas	-	-
Proteobacteria	Caulobacteraceae	Brevundimonas	-	-

Table 5.3 Percentage of relative abundance in organic fertilizer (compost) on *Actinobacteria, Bacteriodetes, Firmicutes* and *Plantomycetes* in 2014 and 2017

Phylum	Related Family	Related Genus	Percentage of relative abundance	
			Compost 2014	Compost 2017
Actinobacteria	Glycomycetaceae	Glycomyces	13	13
Actinobacteria	Pseudonocardiaceae	Jiangella	27	6
Actinobacteria	Pseudonocardiaceae	Thermocrispum	1	4
Actinobacteria	Pseudonocardiaceae	Pseudonocardia	1	2
Actinobacteria	Mycobacteriaceae	Mycobacterium	-	2
Actinobacteria	Euzebyaceae	Euzebya	2	1
Actinobacteria	Nocardiopsaceae	Thermobifida	2	-
Actinobacteria	Brevibacteriaceae	Brevibacterium	-	1
Actinobacteria	Pseudonocardiaceae	Prauserella	-	1
Actinobacteria	Streptomycetaceae	Streptomyces	-	1
Actinobacteria	Nocardioidaceae	Nocardioides	-	-
Actinobacteria	Pseudonocardiaceae	Saccharomonospora	-	-
Actinobacteria	Nocardioidaceae	Actinopolymorpha	-	-
Bacteroidetes	Sphingobacteriaceae	Parapedobacter	2	4
Bacteroidetes	Rikenellaceae	Blvii28	-	2
Bacteroidetes	Rhodothermaceae	Rubricoccus	-	1
Bacteroidetes	Sphingobacteriaceae	Sphingobacterium	-	1
Bacteroidetes	Cryomorphaceae	Cryomorpha	-	-
Bacteroidetes	Sphingobacteriaceae	Olivibacter	-	-
Firmicutes	Bacillaceae	Bacillus	6	2
Firmicutes	Thermoactinomycetaceae	Planifilum	1	-
Firmicutes	Staphylococcaceae	Staphylococcus	-	1
Firmicutes	Bacillaceae	Geobacillus	-	-
Firmicutes	Bacillaceae	Halobacillus	-	-
Firmicutes	Planococcaceae	Solibacillus	-	-
Firmicutes	Planococcaceae	Lysinibacillus	-	-
Planctomycetes	Pirellulaceae	planctomycete	1	5
Planctomycetes	Planctomycetaceae	Planctomyces	1	2

5.3.2 Vegetative growth in immature oil palm

To determine the extent of plant growth, measure vegetative and generative development in immature indices of palms. Not significantly different $p<0.05$ on vegetative development was noted in this research (Table 5.4). Table 5.5 shows significantly difference on frond, canopy and chlorophyll. Dry weight of frond of treatment 1(100 % inorganic fertilizer: 0 % organic fertilizer) and 2 (50 % inorganic fertilizer: 50 % organic fertilizer) of the same value 1.32 cm. Result on canopy showed the highest value of treatment 1 (291.31 cm) followed by treatment 2 (281.06 cm), treatment 4 (279.05 cm) and last treatment 3 (276.05 cm). This research is in line with the work of Saeed *et al.* 2015 that the combination of inorganic fertilizer and organic fertilizer therapy had an important impact and enhanced cucumber yield and development characteristics. Figure 5.12 shows one month after transplanting, Figure 5.13 four months after transplanting, Figure 5.14 - 24 months after transplanting, Figure 5.15 – 36 months after transplanting, Figure 5.16 – 48 months after transplanting, and Figure 5.17 – 60 months after transplanting of oil palm plantation at FELD at Serting Hilir Negeri Sembilan. Application of 50 % inorganic fertilizer; 50 % organic fertilizer, 25 % inorganic fertilizer: 75 % organic fertilizer and 100 % organic fertilizer root can absorb nutrients as well as 100 % inorganic fertilizer.

5.3.3 Foliar

Table 5.6 summarizes data on the measurement of foliar nutrient and Table 5.8 coefficient of phenotypic correction foliar in oil palm plantation. Foliar concentration, there were substantial different in $p<0.05$ magnesium, sulfur and zinc. Foliar P concentration 0.16 cmol(+)/kg on treatment 75 % inorganic fertilizer : 25 % organic

fertilizer at 48 months and the rest of treatment with same valued 0.17 cmol(+)/kg. There has been a major interaction influencing the nutrient concentration of the foliar on P, K, S, Ci, Fe and Zn usually increasing them. The inorganic fertilizer impact on foliar increases P, K and Mg. Table 5.8 show potassium decreased on production of yield for every month. In all treatments 1, 2, 3, and 4 values of P were between 1.46-1.58 cmol(+)/Kg on 12 months, 1.63-1.72 cmol(+)/Kg on 24 months, decreased between 1.39-1.40 cmol(+)/Kg on 48 months and decrease 0.99-1.13 cmol(+)/Kg. K+ was added to increased cell turgor pressure during fiber elongation (Yang *et al.* 2016). In addition, in the upper fruiting branches, the low K− tolerant genotype to have K+ absorptive capacity (Yang *et al.* 2016). The function of potassium is known to effect on yield production. Similarly leaf K, Ca, Mg and boron level also showed quite small different among the treatments and are adequate for optimum palm growth. This suggests that only leaf N was the limiting factor, which probably affected the oil palm growth and yield.

Table 5.4 Mean squares of vegetative growth of oil palm plantation

Factors	DF	Number of Leaflet	Thick of Leaflet	Length of Leaflet	Width of Leaves	Thick of Leaves	Canopy	PCS	Leaves Area	Dry Weight /Frond	Chlorophyll
Block	3	ns	ns	ns	ns	*	ns	ns	ns	ns	ns
Treatment	3	ns	ns	ns	ns	ns	*	ns	ns	*	*
Months	4	***	***	***	***	***	***	***	***	***	***
Treatment*Months	12	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

For each factor, means within a column followed by ns letter are not significantly different by Tukey Test at $P \geq 0.05$, * = significant and $P \leq 0.05$, respectively. ** = significant at $P \leq 0.001$ and * = significantly at $P \leq 0.0001$**

Table 5.5 Dry weight of frond, canopy and chlorophyll content in oil palm plantation

Treatments	Dry weight of frond (cm)	Canopy (cm)	Chlorophyll
100 % Inorganic Fertilizer : 0 % Organic Fertilizer	1.34a	291.31a	72.27a
50 % Inorganic Fertilizer : 50 % Organic Fertilizer	1.34a	281.06ab	72.14a
25 % Inorganic Fertilizer : 75 % Organic Fertilizer	1.26ab	276.05b	69.4ab
0 % Inorganic Fertilizer : 100 % Organic Fertilizer	1.23b	279.05ab	70.97b

For each factor, means within a column followed by ns letter are not significantly different by Tukey Test at $P \geq 0.05$, * = significant and $P \leq 0.05$, respectively. ** = significant at $P \leq 0.001$ and * = significantly at $P \leq 0.0001$**

Table 5.6 Mean squares of foliar growth of oil palm plantation

Factors	DF	Total Nitrogen (%)	cmol(+)/Kg				mg/kg					
			P	K	Ca	Mg	B	S	CI	Cu	Fe	Zn
Block	3	**	ns	ns	ns	ns	ns	ns	ns	ns	*	ns
Treatment	3	ns	ns	ns	ns	*	ns	*	ns	ns	ns	*
Months	1	***	***	***	***	***	***	***	***	***	***	***
Treatment*Months	4	ns	**	*	ns	ns	ns	*	*	ns	*	*

For each factor, means within a column followed by ns letter are not significantly different by Tukey Test at $P \geq 0.05$, * = significant and $P \leq 0.05$, respectively. ** = significant at $P \leq 0.001$ and * = significantly at $P \leq 0.0001$**

Table 5.7 Coefficient of phenotypic correlation foliar in oil palm plantation

	Number of leaves	Thick	Length	Width	Canopy	PCS	Leaf Area	Dry Weight Frond	Chlorophyll
Number of leaves	1								
Thick	0.76***	1							
Length	0.98***	0.78***	1						
Width	0.82***	0.41**	0.83***	1					
Canopy	0.98***	0.72***	0.98***	0.86***	1				
PCS	0.97***	0.82***	0.97***	0.83***	0.95***	1			
Leaf Area	0.96***	0.71***	0.97***	0.86***	0.98***	0.93***	1		
Dry Weight Frond	0.96***	0.81***	0.97***	0.83***	0.96***	0.98***	0.96***	1	
Chlorophyll	0.43***	0.38**	0.41**	0.35**	0.39**	0.47***	0.40**	0.45***	1

For each factor, means within a column followed by ns letter are not significantly different by Tukey Test at $P \geq 0.05$, * = significant and $P \leq 0.05$, respectively. ** = significant at $P \leq 0.001$ and * = significantly at $P \leq 0.0001$**

Table 5.8 Mean of P on foliar of oil palm plantation

Treatment	Months	K cmol(+)/Kg
100 % Inorganic Fertilizer : 0 % Organic Fertilizer	12	1.58abc
	24	1.66ab
	36	1.41de
	48	0.99f
	60	1.34f
50 % Inorganic Fertilizer : 50 % Organic Fertilizer	12	1.56abcd
	24	1.63abc
	36	1.39e
	48	0.98f
	60	1.35f
75 % Inorganic Fertilizer : 25 % Organic Fertilizer	12	1.46cde
	24	1.71a
	36	1.40de
	48	1.06f
	60	1.34f
0 % Inorganic Fertilizer : 100 % Organic Fertilizer	12	1.52bcde
	24	1.72a
	36	1.40de
	48	1.13f
	60	1.383fe

For each factor, means within a column followed by ns letter are not significantly different by Tukey Test at $P \geq 0.05$, * = significant and $P \leq 0.05$, respectively. ** = significant at $P \leq 0.001$ and * = significantly at $P \leq 0.0001$.**

5.3.4 Soil physicochemical properties

For all the soil nutrients tested, there was no significant difference between treatments as shown in Table 5.9. As a result, replacing organic fertilizer with the same nutrient with inorganic fertilizer implementation with four applications per year had no negative effect on oil palm nutrition. Even in the 100 % organic fertilizer substitute, there was no significant difference in the level of plant nutrient between 100 % implementation of inorganic fertilizer. From a financial point of view, the application of EFB was very cost effective considering the rise in output achieved. More of the applied N and K fertilized were taken up owing to reduced ground wash and leaching losses.

5.3.5 Microbial diversity

Figure 5.10 show the effect of different treatments of fertilizer on microbial diversity. Treatment 1 (100 % inorganic fertilizer) showed microbial diversity decreased in 2017 compared to 2014. Interesting result on treatment1 increased microbial diversity year 2017 on unfertilized area at 15-50 cm depth. Treatment 2 (50 % inorganic fertilizer: 50 % organic fertilizer) and treatment 3 showed increased microbe diversity especially at fertilizer area with depth 0-15 cm. This is different with treatment 4 (100 % organic fertilizer) which showed level of microbes was already low in 2014, was high in fertilizer area 0-15 in 2017. The results showed that the application of mixed inorganic/compost fertilizer increased the abundance of soil microbes. The dominant phyla across all samples were *Firmicutes, Proteobacteria, Bacteroidetes, Actinobacteria, Acidobacteria, Planctomycetes, Verrucomicrobia*, and *Chloroflexi* (the total relative abundances of each phylum>10 %), accounting for more than 95 % of the bacterial sequences. The effect of inorganic fertilizer on genus is shown Table

5.10 and 5.11. Table 5.10 shows result on genus, kingdom on archaea, bacteria on *Firmicutes* and *Proteobacteria.* Archaea on phylum *Crenarchaeota* and genus *Nitrosotalea* appeared after four years application with mixed (50 % and 75 %) inorganic fertilizer and 100% inorganic fertilizer. From the previous research as discussed in objective one, archaea was only present in secondary forest soil. Application of inorganic fertilizer effect phylum *Firmicutes* increased abundance of microbe on genus from 2014 and 2017. The dominant genus were *Firmicutes*, *Alicyclobacillus* and *Bacillus*, followed by *Proteobacteria, Burkholderia* and *Rhodoplanes.* Increased abundance of microbes in 2017 with mixed (50 % and 75 %) inorganic fertilizer and 100 % inorganic fertilizer. Phylum *Bacteroidetes* with genus *Flavobacterium* third highest after *Firmicutes* and *Proteobacteria.* Figure 5.18, Treatment 1 as 100 % inorganic fertilizer: 0 % organic fertilizer dominant phylum *Proteobacteria.* Same result treatment 3 (75 % inorganic fertilizer: 25 % organic fertilizer) and treatment 4 (0 % inorganic fertilizer: 100 % organic fertilizer high phylum *Proteobacteria* after four years application fertilizer. Interested funding at treatment 2 as 50 % inorganic fertilizer: 50 % inorganic fertilizer higher phylum *Firmicutes.* The impacts of different fertilizations on bacterial communities have been reported by previous studies on paddy soil (Geisseler *et al.* 2017).

Table 5.9 Mean square of soil on oil palm plantation

Factors	DF	pH H2O (1:2.5)	Total Nitrogen (%)	Organic Carbon (%)	P (mg/kg) Total	P (mg/kg) Avail	C.E.C (cmol(+)/Kg)	Exch cmol(+)/Kg K	Exch cmol(+)/Kg Ca	Exch cmol(+)/Kg Mg	Exch cmol(+)/Kg Al	mg/kg B	mg/kg Mn	mg/kg Fe	mg/kg Zn
Block	3	ns	**	*	***	***	***	***	***	***	ns	***	**	***	***
Treatment	3	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Depth	1	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns
Years	1	ns	***	***	***	***	***	***	***	***	ns	***	***	***	***
Treatment*Depth	3	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Treatment*Years	3	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns
Treatment*Depth*Years	4	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

For each factor, means within a column followed by ns letter are not significantly different by Tukey Test at $P \geq 0.05$, * = significant and $P \leq 0.05$, respectively. ** = significant at $P \leq 0.001$ and * = significantly at $P \leq 0.0001$**

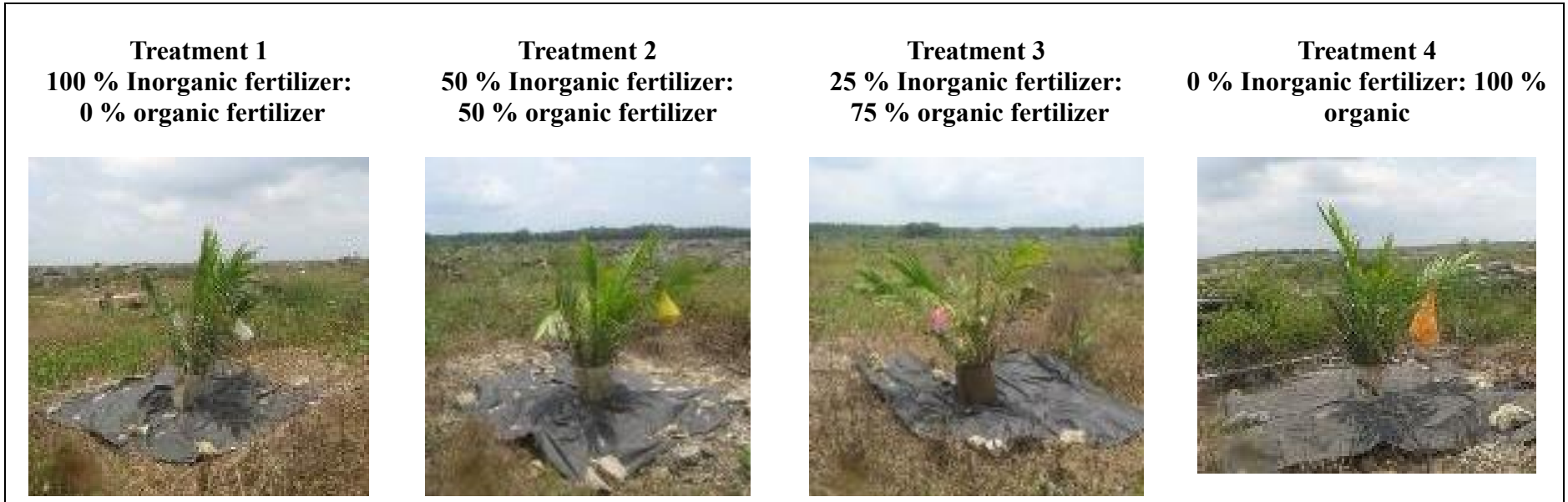


Figure 5.12 One month after transplanting of oil palm plantation at FELDA Serting Hilir Negeri Sembilan

(December 2013)

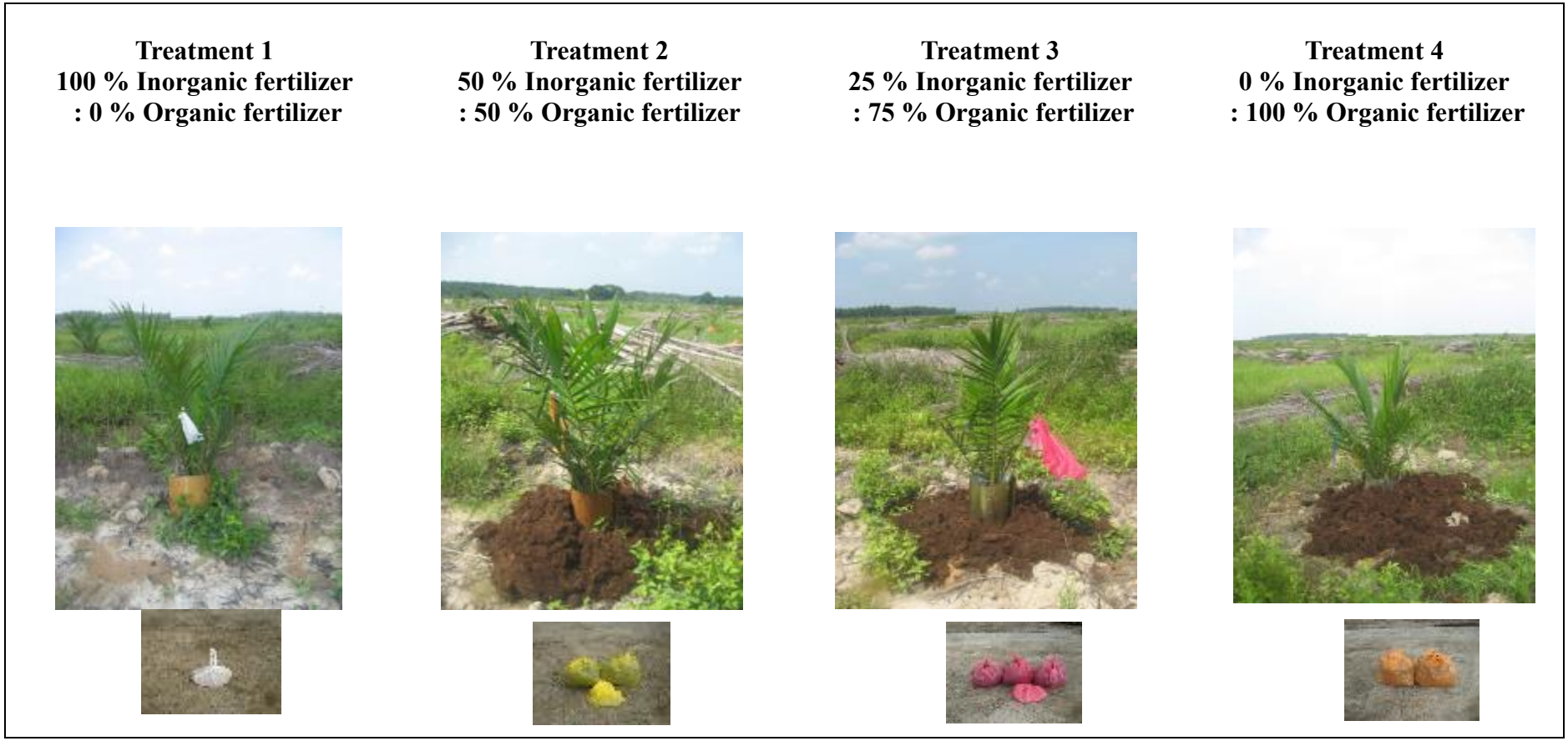


Figure 5.13 12 months after transplanting of oil palm plantation at FELDA Serting Hilir Negeri Sembilan (December 2014 / First year)

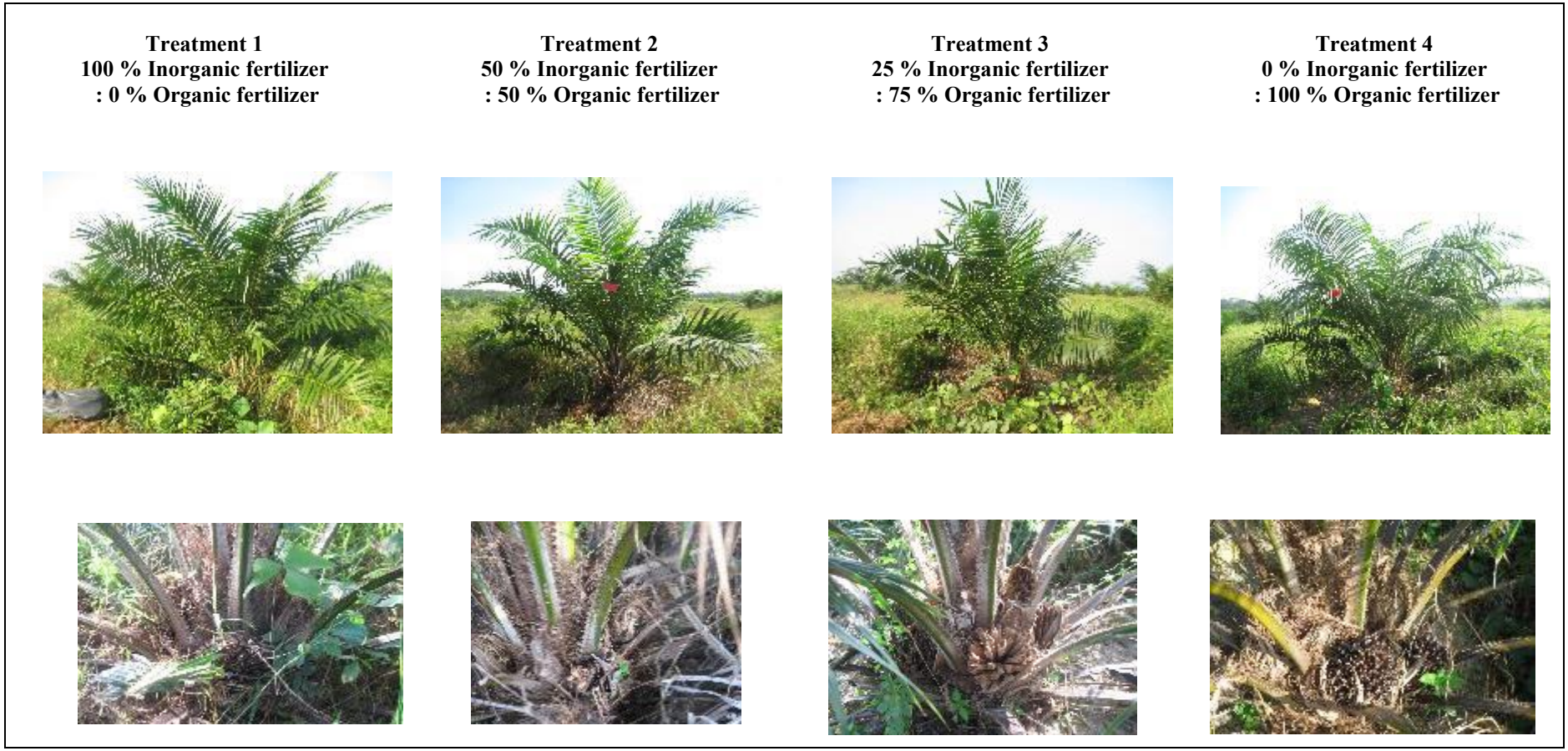


Figure 5.14 24 months after transplanting of oil palm plantation at FELDA Serting Hilir Negeri Sembilan (December 2015 / Second year)

Figure 5.15 36 months plant transplanting of oil palm plantation at FELDA Serting Hilir Negeri Sembilan (December 2016 / Third Year)

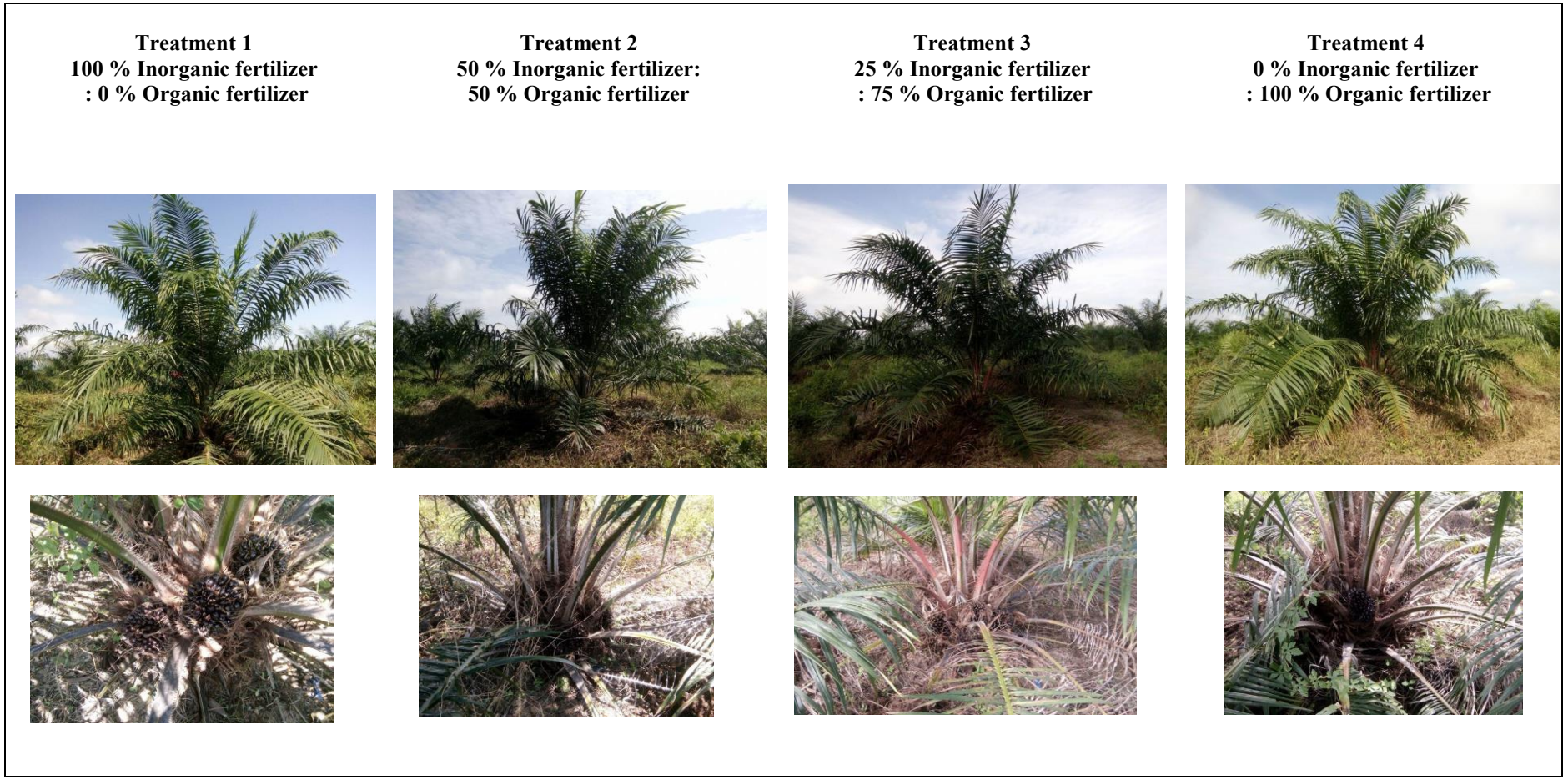


Figure 5.16 48 months plant transplanting of oil palm plantation at FELDA Serting Hilir Negeri Sembilan (December 2017 /Fourth year)

Figure 5.17 60 months plant transplanting of oil palm plantation at FELDA Serting Hilir Negeri Sembilan (December 2018 / Fifth year)

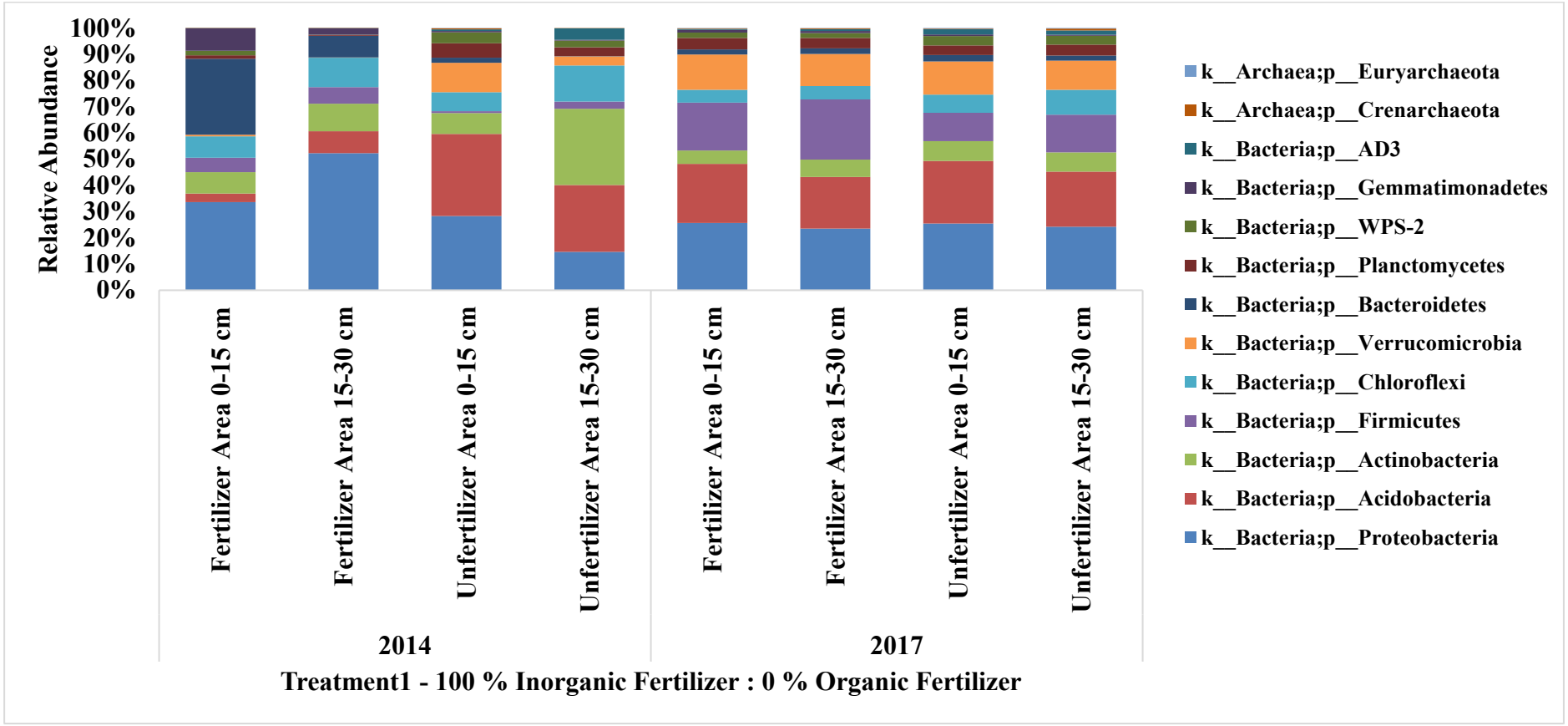


Figure 5.18 Relative abundance of phylum treatment 1 (100 % Inorganic Fertilizer with 0 % Organic Fertilizer) between 2014 and 2017 for oil palm plantation

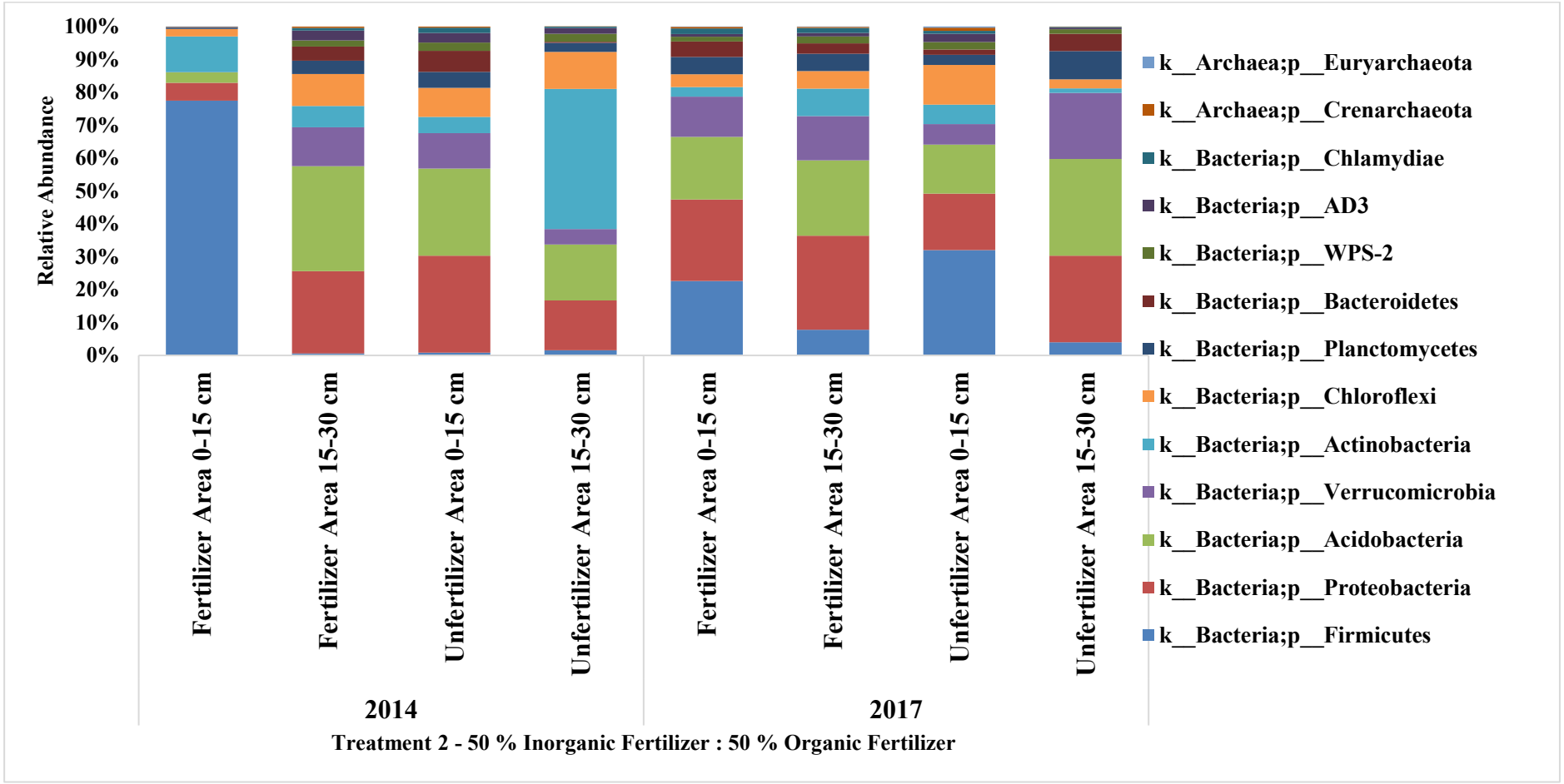


Figure 5.19 Relative abundance of phylum treatment 2 (50 % Inorganic Fertilizer with 50 % Organic Fertilizer) between 2014 and 2017 for oil palm plantation

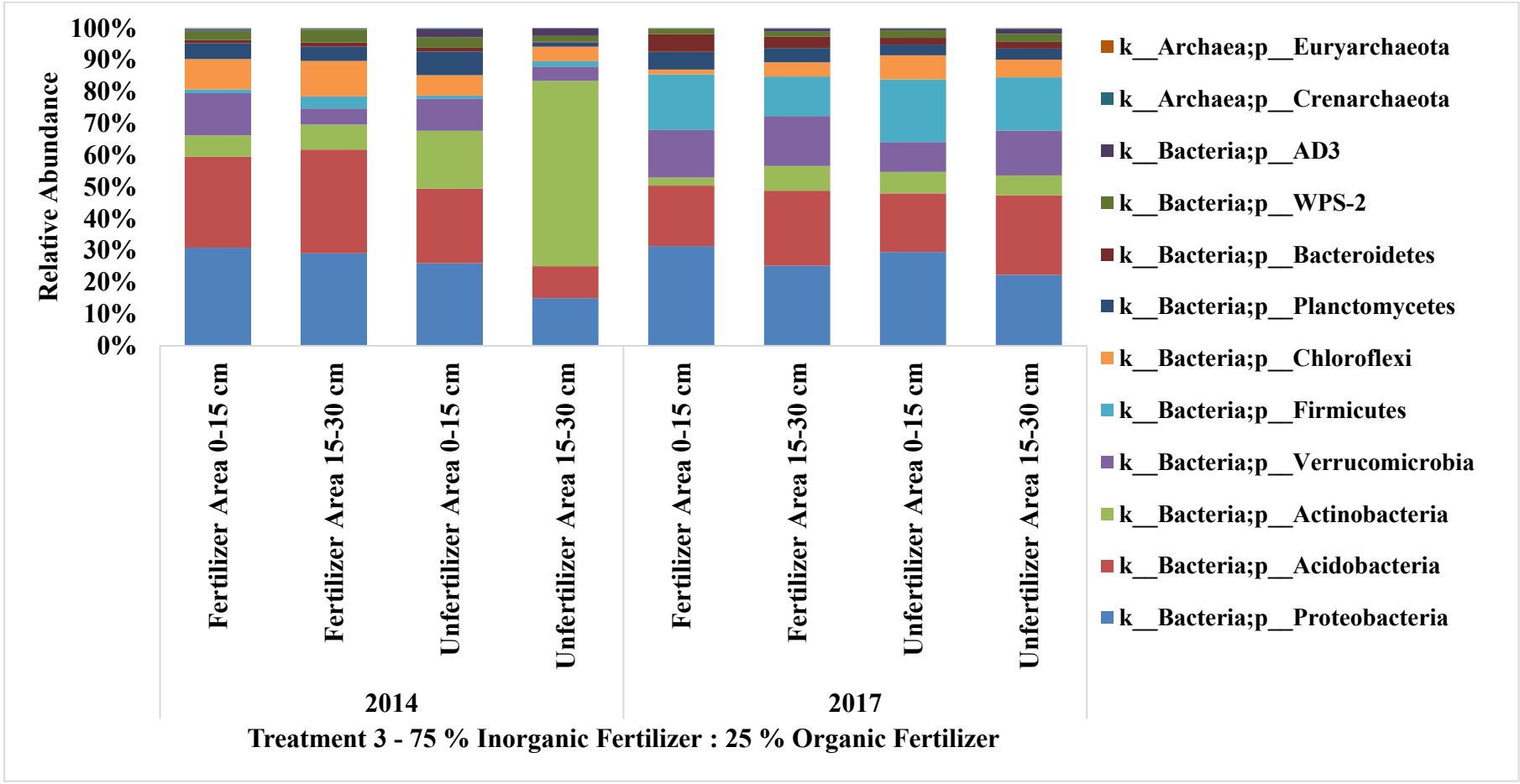


Figure 5.20 Relative abundance of phylum treatment 3 (25 % Inorganic Fertilizer with 75 % Organic Fertilizer) between 2014 and 2017 for oil palm plantation

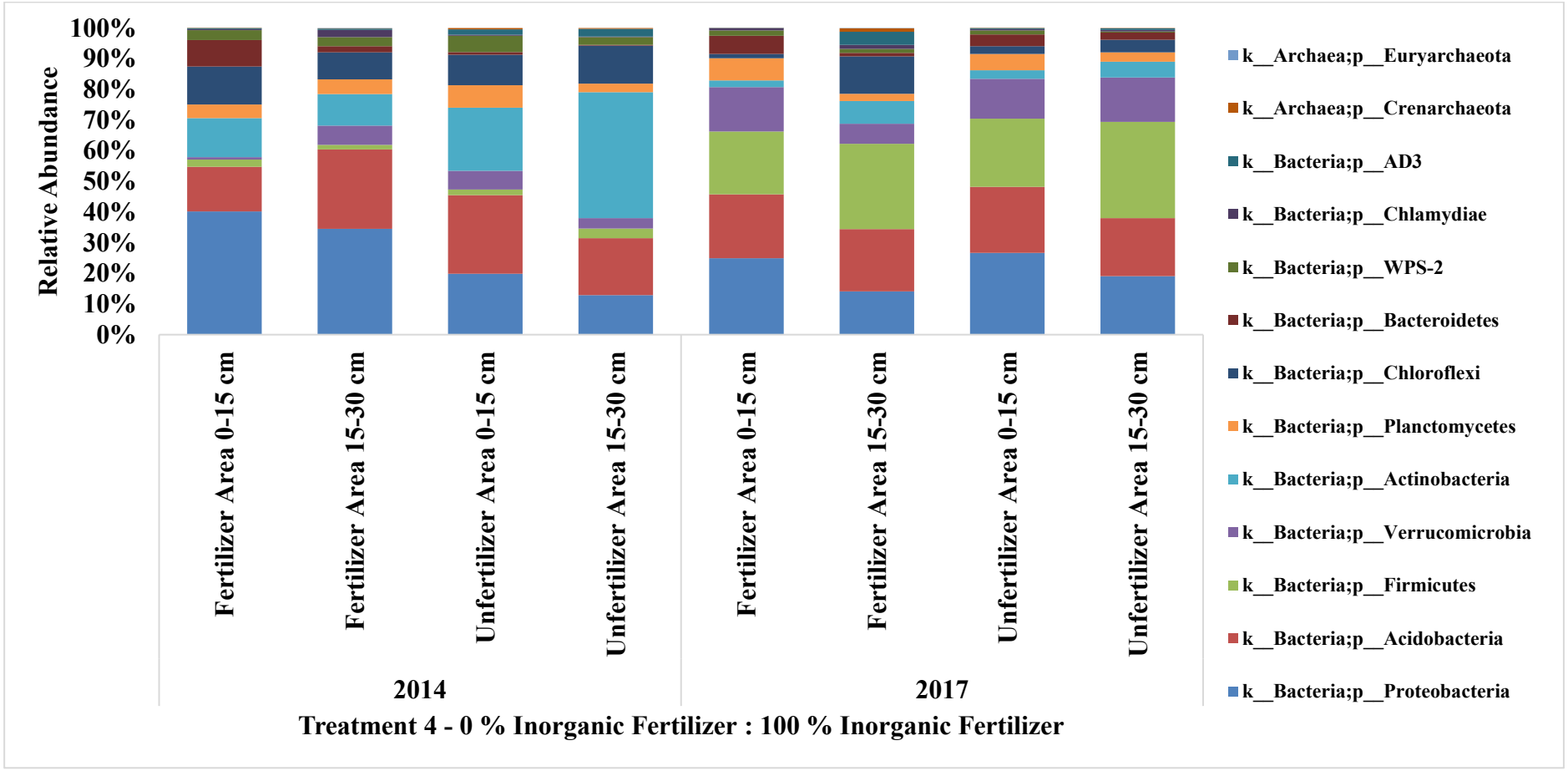


Figure 5.21 Relative abundance of phylum treatment 4 (0 % Inorganic Fertilizer with 100 % Organic Fertilizer) between 2014 and 2017 for oil palm plantation

Table 5.10 Percentage of relative abundance of genus treatment 1, treatment 2, treatmenr3 and treatment 4 at 0-15 cm for oil palm plantation

Kingdom	Phylum	Genus	2014				2017			
			Trt1	Trt2	Trt3	Trt4	Trt1	Trt2	Trt3	Trt4
Archaea	*Crenarchaeota*	*Nitrosotalea*	-	-	-	-	-	0.02	0.04	0.03
Bacteria	*Bacteroidetes*	*Sporocytophaga*	-	-	0.12	0.02	-	0.04	0.13	0.03
Bacteria	*Bacteroidetes*	*Flavobacterium*	-	-	-	-	0.24	0.84	1.60	0.39
Bacteria	*Bacteroidetes*	*Flavihumibacter*	-	-	-	-	-	0.02	0.08	0.04
Bacteria	*Bacteroidetes*	*Flavisolibacter*	0.74	-	-	-	-	0.02	0.04	-
Bacteria	*Firmicutes*	*Alicyclobacillus*	1.07	34.29	0.21	0.24	10.85	5.11	1.91	0.80
Bacteria	*Firmicutes*	*Bacillus*	1.07	3.71	0.47	1.26	5.07	5.11	5.28	3.95
Bacteria	*Firmicutes*	*Caloramator*	-	0.02	-	-	-	-	0.01	0.01
Bacteria	*Firmicutes*	*Lactococcus*	-	-	-	-	-	0.02	0.02	0.03
Bacteria	*Firmicutes*	*Clostridium*	0.04	0.18	0.07	0.01	0.44	6.88	5.27	0.03
Bacteria	*Acidobacteria*	*Edaphobacter*	-	-	-	-	0.44	0.65	0.44	0.19
Bacteria	*Acidobacteria*	*Candidatus Koribacter*	0.10	-	0.54	0.37	0.99	0.96	0.78	0.64
Bacteria	*Acidobacteria*	*Candidatus Solibacter*	0.82	0.06	3.56	0.59	1.78	1.79	1.45	1.72
Bacteria	*Actinobacteria*	*Sinomonas*	0.19	-	-	0.11	0.23	0.15	0.07	1.72
Bacteria	*Actinobacteria*	*Mycobacterium*	0.18	0.37	0.78	0.80	0.15	0.04	-	0.04
Bacteria	*Actinobacteria*	*Streptacidiphilus*	0.03	-	-	0.11	0.79	0.36	0.56	0.34
Bacteria	*Actinobacteria*	*Streptacidiphilus*	0.82	-	-	0.38	0.19	0.04	0.11	0.20

Table 5.11 Percentage of relative abundance of genus treatment 1, treatment 2, treatmenr3 and treatment 4 at 0-15 cm depth for oil palm plantation

Kingdom	Phylum	Genus	2014				2017			
			Trt1	Trt2	Trt3	Trt4	Trt1	Trt2	Trt3	Trt4
Bacteria	*Proteobacteria*	*Rhodoplanes*	0.64	0.03	4.67	1.52	1.06	0.92	1.65	1.18
Bacteria	*Proteobacteria*	*Reyranella*	0.16	-	0.04	0.02	0.38	0.42	0.25	0.11
Bacteria	*Proteobacteria*	*Salinispora*	-	-	-	0.06	0.09	0.05	0.65	0.12
Bacteria	*Proteobacteria*	*Geobacter*	-	-	-	-	-	0.23	0.31	0.01
Bacteria	*Proteobacteria*	*Acinetobacter*	-	-	-	-	-	0.06	0.12	0.07
Bacteria	*Proteobacteria*	*Pseudomonas*	-	-	-	0.21	-	0.16	-	0.07
Bacteria	*Proteobacteria*	*Steroidobacter*	-	-	-	0.23	0.03	0.23	0.07	0.03
Bacteria	*Proteobacteria*	*Phenylobacterium*	0.38	-	-	0.01	-	0.11	0.22	0.02
Bacteria	*Proteobacteria*	*Pedomicrobium*	-	-	-	0.02	-	0.01	0.03	0.035
Bacteria	*Proteobacteria*	*Rhodoblastus*	-	-	0.03	-	-	0.25	0.40	0.14
Bacteria	*Proteobacteria*	*Mesorhizobium*	-	-	-	-	-	0.06	0.03	-
Bacteria	*Proteobacteria*	*Rhizobium*	-	-	-	-	0.03	0.02	0.03	0.03
Bacteria	*Proteobacteria*	*Reyranella*	0.16	-	0.04	0.02	2.25	0.02	0.25	0.11
Bacteria	*Proteobacteria*	*Burkholderia*	0.06	-	0.06	0.06	2.25	2.95	2.94	2.53
Bacteria	*Proteobacteria*	*Bdellovibrio*	-	-	-	0.05	0.16	0.08	0.05	0.09
Bacteria	*Proteobacteria*	*Syntrophobacter*	-	-	-	-	-	0.04	0.02	-
Bacteria	*Proteobacteria*	*Aquicella*	-	-	1.00	0.02	0.83	0.87	0.16	0.97
Bacteria	*Proteobacteria*	*Dyella*	0.22	0.30	0.16	0.90	0.15	0.05	-	0.18

5.3.6 Oil yield

5.3.6.1 Effect of fertilizer treatments on FFB Yield

Figure 5.12 shows fresh fruit bunch (FFB) between treatments 1, 2, 3, and 4. Figure 5.20 shows comparison of FFB and mesocarp between treatments 1, 2, 3, and 4. Table 5.12 shows that data after 4 years were not significantly different in bunch number (BN), bunch weight (BW) and average bunch weight (ABW). The highest average bunch weight (AWB) for 2018 7.64 on treatment1, followed by treatment 2 (5.97), treatment 4 (5.87) and treatment 3 (5.73). The highest production in tonnes for 2018 was in treatment 1 (17.90), followed by treatment 4 (16.20), treatment 2 (13.00) and treatment 3 (12.40). The FFB yield in the first and second years of the experiment were omitted to ensure that the response obtained was from the effect for fertilizer treatments, and to minimize the residual effect from the application prior to the implementation of the project. Table 5.13 shows data for oil extraction rate (OER), fresh fruit bunch (FFB) and crude palm oil (CPO) for 2017 and 2018 of oil palm plantation. Oil extraction rate (OER) for 2017, 20.50 % (treatment 1), 19.04 % (treatment2), 16.63 % (treatment 3), and 16.29 % (treatment4). Fresh fruit bunch (FFB) 2017, 69.13 t ha $^{-1}$yr^{-1} for treatment 1, 68.63 t ha $^{-1}$yr^{-1} for treatment 2, 67.7 t ha $^{-1}$yr^{-1} for treatment 3, and 67.04 t ha $^{-1}$yr^{-1} for treatment 4. The potential of crude palm oil (CPO) for 2017 main by treatment1 (1416.82), treatment 2(1306.63), treatment3 (1126.00) and treatment 4 (1092.40). Oil extraction rate (OER) for 2018 showed similar data between 24.62 % - 26.05 % for treatment. Same with fresh fruit bunch (FFB), between 54.37 t ha $^{-1}$yr^{-1} to 59.65 t ha $^{-1}$yr^{-1}. And crude palm oil, 1380 to 1553.57. The number of OER and FFB for 2018 were quite similar for the consecutive

years. A similar upward trend in the CPO yield in the second years harvested was observed in the oil palm plantation surrounding the experimental area land was probably due to prevailing rainfall pattern effect on absorption of nutrient on soil effect yield.

5.3.6.2 Oil extraction rate (OER)

Figures 5.22 and 5.23 show comparison between fresh fruit bunch (EFB) and mesocarp between treatment 1, treatment 2, treatment 3 and treatment 4. Although there are many factors affecting the measurement of OER, such as crop weight, cage weight, and assuming that the figures submitted by the mills are reliable as mills are subjected to regular enforcement work carried out by FELDA. Figures 5.24 and 5.25 show oil to bunch and oil to kernel from FFB yield recorded for first year harvested 2017 and second years harvested 2018. The oil to bunch for 2017 decreased among the treatment. Treatment 1 as commercial had 23.03%, followed by treatment 2 had 21.44 %, then treatment 3 had 18.73 % and treatment 4 had 18.35 % of oil percentage. Data for oil to kernel in 2017 showed that treatment 4 (100 % inorganic fertilizer) contained high oil to bunch 6.81% compared with other treatments. Other treatment on oil kernel showed data are similar, Treatment 1 (5.99 %), teratment2 (6.06 %) and treatment 3 (5.29 %). The oil to bunch and oil to kernel for 2018 recorded as second year's production showed not significantly different. Treatment 1 (100% inorganic fertilizer) valued 27.73%, treatment 2 (50 % inorganic fertilizer: 50 organic fertilize) 27.39 %), treatment 3 (25 % inorganic fertilizer: 75 % organic fertilizer) at 29.33 % and treatment 4 (100 % organic fertilizer) at 27.80 %. Result on oil to kernel for 2018, Treatment1 (100 % inorganic fertilizer) 4.92 %, treatment 2 (50 % inorganic fertilizer: 50 % organic fertilize) 4.99 %, treatment 3 (25 % inorganic fertilizer: 75 % organic

fertilizer) 4.97 % and treatment 4 (100 % organic fertilizer) 5.58 %. Data in 2018 proved that application of mixed inorganic fertilizer even 100 % inorganic fertilizer did not effect oil production of oil palm plantation. The first step in the milling of oil palm fruits is the production of CPO and palm kernel (PK). OER and kernel extraction rate (KER) are two important parameters that are directly related to the profitability of an oil palm enterprise. The main objective of the mills is to contribute to the attainment of as high as possible on these two extraction rates in order to achieve maximum production of CPO and kernel per hectare. The CPO produced is a function of the quantity/quality of the fresh fruit bunches (FFB) milled and the OER of the mills. Support data, as the fruitlet grows, maximum accumulations of oil components known as triacylglycerols replace the chlorophyll in the mesocarp. Chlorophyll plays an important role in synthesizing carbohydrates in the fruitlet.

5.3.7 Economic statistical analysis

The statistical analysis model for tracking input and output flows was developed using Excel (Microsoft Inc., Redmond, Ca). Data on the farming methods used for the management of oil palm tree plantations were gathered from the literature and used to model the planting life cycle operations, planting maintenance, FFB harvesting, FFB transport, storage, loading and transportation of main biomass to the biorefinery. Table 5.14 shows in (Treatment 1-100 % inorganic fertilizer), Table 5.15 (Treatment 2 - 50 % inorganic fertilizer: 50% organic fertilizer), Table 5.16 (Treatment 3 - 25 % inorganic fertilizer: 75 % organic fertilizer), and Table 5.17 (0 % inorganic fertilizer: 100% organic fertilizer), from 2014 until 2018 for per palm, per acre and per hectare of oil palm plantation. In this result show cost per palm for 2014, RM5.99 (Treatment 1) and RM17.82 (Treatment 2). By the way cost per 2018, RM10.53 (Treatment1) and

RM13.18 (Treatment2). Cost of production for per acre for 2018 was RM579.15 (Treatment1) and RM724.9 (Treatment2). And cost per hectare 2018, RM1421.55 (Treatment 1) and RM1779.3 (Treatment 2). Table 5.15 shows in treatment 3 (25 % inorganic fertilizer: 75 % organic fertilizer) and Treatment 4 (100 % organic fertilizer) from 2014 until 2018 for per palm, per acre and per hectare of oil palm plantation. Reduction cost of per palm Treatment 3 shows in 2018 was RM13.34 compared to Treatment 4 cost around RM15.38. Cost per acre and per hectare for treatment 4 as 100 % inorganic fertilizer high compared to another treatment. Composition with cost of Treatment 1 (RM1421.55) and Treatment 2 (RM1779.30) on per hectare different RM 357.7 are comparable. If 50 % inorganic fertilizer: 50 % organic fertilizer is used, it can save cost of imported inorganic fertilizer (Figure 5.26).

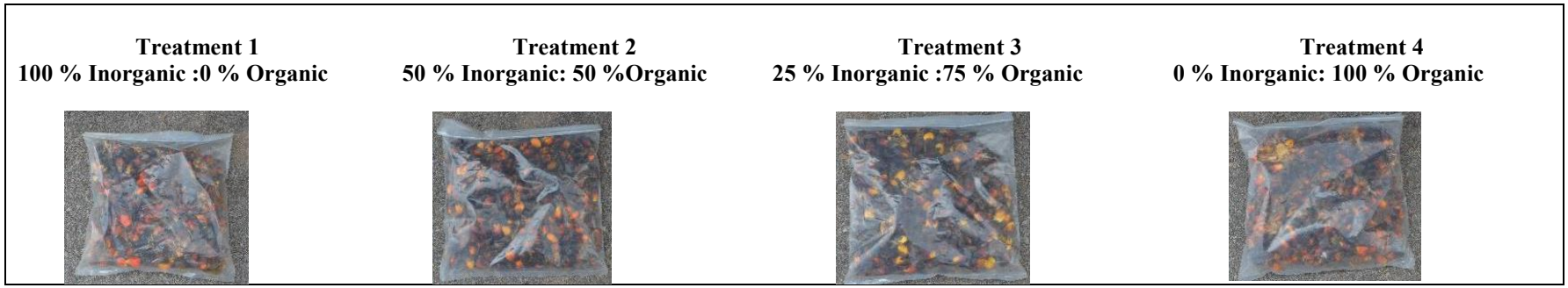


Figure 5.22 Fresh fruit bunch (FFB) between treatment 1, treatment 2, treatment 3 and treatment 4 at FELDA Serting Hilir

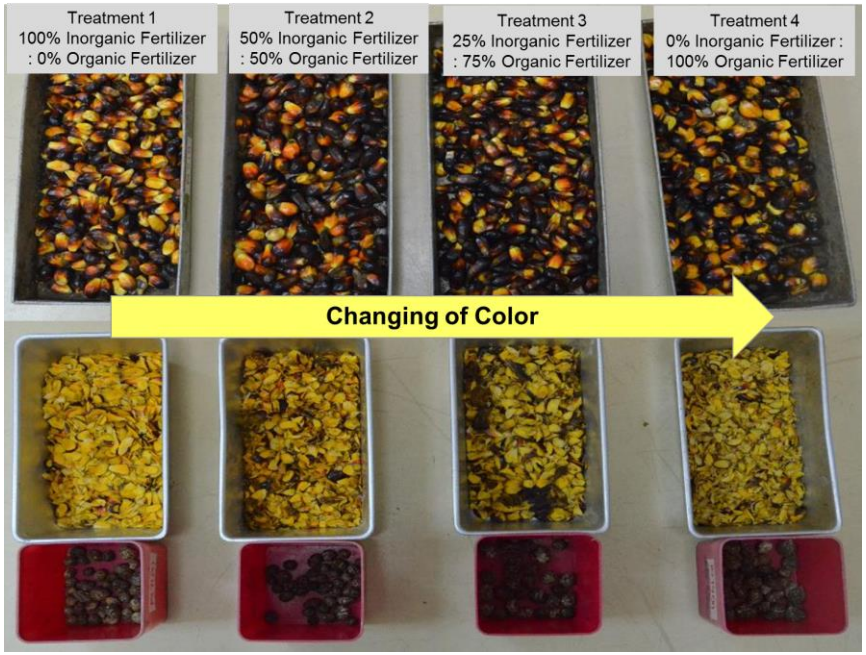


Figure 5.23 Comparison fresh fruit bunch (FFB) and mesocarp between treatment 1, treatment 2, treatment 3 and treatment 4 at FELDA Serting Hilir Negeri Sembilan

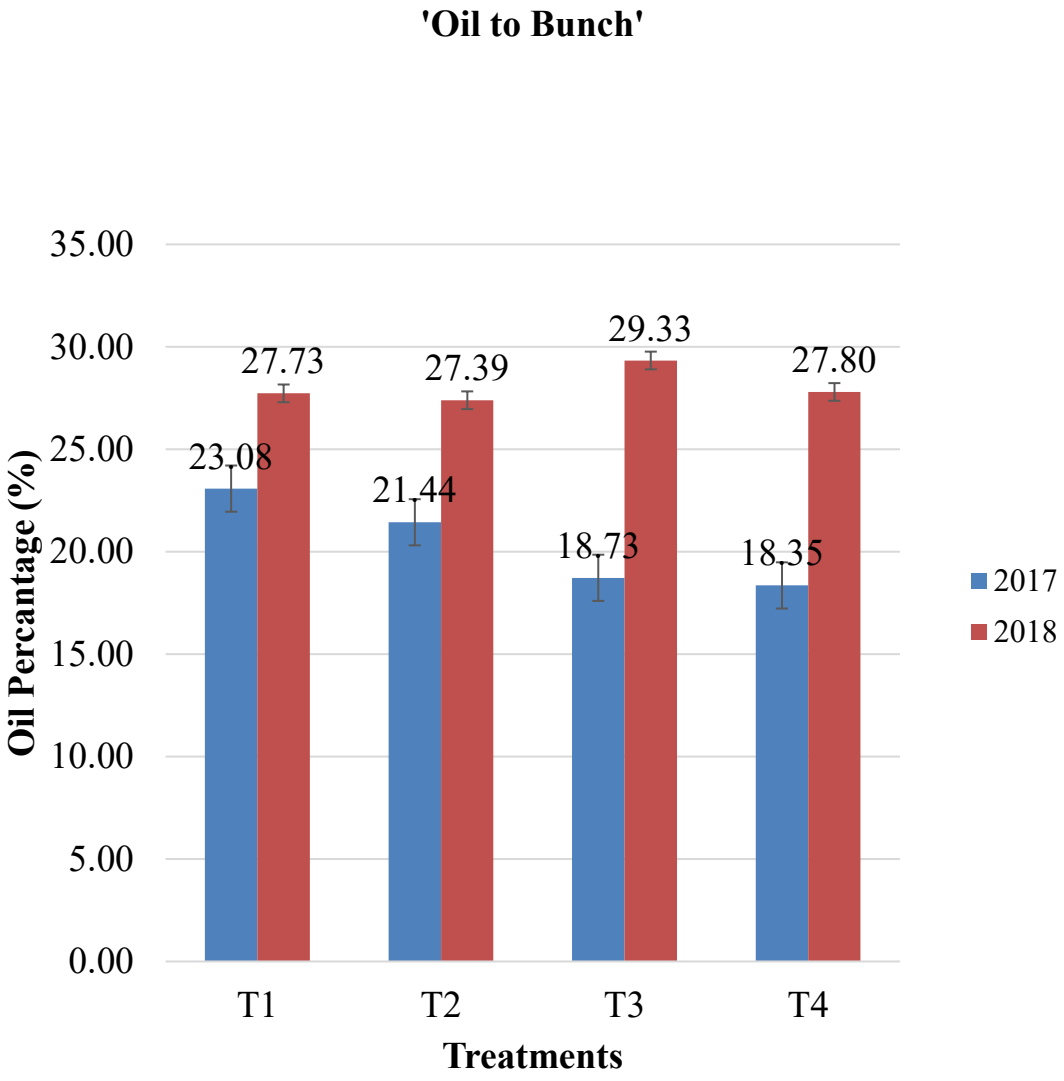


Figure 5.24 Comparison oil to bunch between treatment 1, treatment 2, treatment 3 and treatment 4

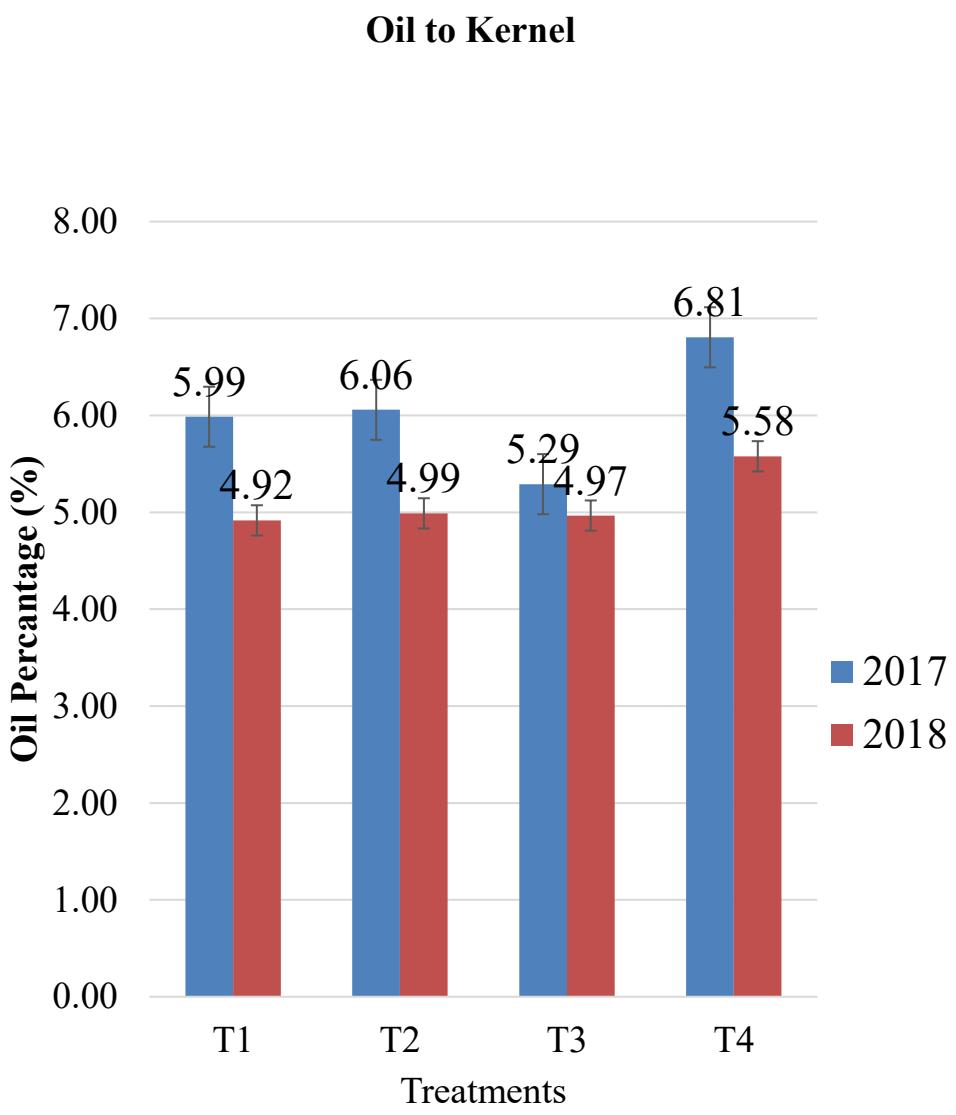


Figure 5.25 Comparison oil to kernel between Treatment 1, Treatment 2, Treatment 3 and Treatment 4

Table 5.12 Bunch number (BN), bunch weight (BW) and average bunch weight (ABW) for 2017 and 2018 of oil palm plantation

Treatment	Bunch number (BN)		Bunch Weight (BW)		Average Bunch Weight (ABW)		Ton	
	2017	2018	2017	2018	2017	2018	2017	2018
T1	15.94a	6.41a	48.80a	52.02a	1.43a	7.64a	7.23a	17.9a
T2	14.30a	5.39a	48.79a	37.59a	1.28a	5.97ab	7.22a	13.0ab
T3	11.67a	5.82a	34.64a	37.97a	1.23a	5.73ab	5.13a	12.4b
T4	10.73a	5.97a	33.46a	44.64a	0.67a	5.87b	4.95a	16.2ab

Table 5.13 Oil extraction rate (OER), fresh fruit bunch (FFB), and crude palm oil (CPO) for 2017 and 2018 of oil palm plantation

Treatments	Year	Values		
		Oil Extraction Rate % (OER)	Fresh Fruit Bunch ($t\ ha^{-1}yr^{-1}$)(FFB)	Crude Palm Oil (millstones) (CPO)
T1	2017	20.50	69.13	1416.82
	2018	24.62	56.6	1393.23
T2	2017	19.04	68.63	1306.63
	2018	24.32	54.37	1322.41
T3	2017	16.63	67.7	1126.00
	2018	26.05	59.65	1553.59
T4	2017	16.29	67.04	1092.40
	2018	24.69	55.91	1380.22
Mean		21.52	62.38	1323.91

Table 5.14 Cost of production (CPO) for treatment 1 on 2014 - 2018 for per palm, per acre, and per hectare of oil palm plantation

100% Inorganic Fertilizer: 0% Inorganic Fertilizer															
Fertilizer	**Year of trial (kg/palm)**					**Year or trail (kg/acre)**					**Years of trail / (kg / hectare)**				
Years	**2014**	**2015**	**2016**	**2017**	**2018**	**2014**	**2015**	**2016**	**2017**	**2018**	**2014**	**2015**	**2016**	**2017**	**2018**
Sulphate of ammonia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rock phosphate	0.50	-	2.00	2.00	2.00	27.5	-	110	110	110	67.5	-	270	270	270
Muariate of potash	-	1.00	-	-	-	-	55	-	-	-	-	135	-	-	-
Kieserite	-	-	1.00	1.00	1.00	-	-	55	55	55	-	-	135	135	135
NPKMg	4.75	6.50	6.75	7.25	7.25	261.25	357.50	371.25	398.75	398.75	261.25	877.5	911.25	978.75	978.75
Organic Fertilizer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cost Material (RM)	5.43	6.50	6.75	9.63	9.63	298.65	357.5	371.25	529.65	529.65	733.05	877.5	911.25	1300.05	1300.05
Cost Application (RM)	0.44	0.56	0.39	0.9	0.9	24.2	30.8	21.45	49.5	49.5	59.4	75.6	52.65	121.5	121.5
Cost Transportation (RM)	0.12	0	0	0	0	6.6	0	0	0	0	16.2	0	0	0	0
Total Cost (RM)	5.99	7.06	7.14	10.53	10.53	329.45	388.3	392.7	579.15	579.15	808.65	953.1	963.9	1421.55	1421.55

Table 5.15 Cost of production (CPO) for treatment 2 on 2014 - 2018 for per palm, per acre, and per hectare of oil palm plantation

50% Inorganic: 50% Organic Fertilizer															
Fertilizer	**Year of trial (kg/palm)**					**1 Acre / 55 palm**					**1 Hectare/ (kg / 135 palms)**				
Years	**2014**	**2015**	**2016**	**2017**	**2018**	**2014**	**2015**	**2016**	**2017**	**2018**	**2014**	**2015**	**2016**	**2017**	**2018**
Sulphate of ammonia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rock phosphate	0.50	-	2.00	2.00	2.00	27.5	-	110	110	110	67.5	-	270	270	270
Muariate of potash	-	1.00	-	-	-	-	55	-	-	-	-	135	-	-	-
Kieserite	-	-	1.00	1.00	1.00	-	-	55	55	55	-	-	135	135	135
NPKMg	2.50	3.26	3.39	3.63	3.63	137.5	179.3	186.45	199.65	199.65	337.5	440.1	457.65	490.05	490.05
Organic Fertilizer	11.25	28.80	31.32	17.07	17.07	618.75	1584	1722.6	938.85	938.85	1518.75	3888	4228.2	2304.45	2304.45
Cost Material (RM)	16.60	14.90	16.42	11.69	11.69	913	819.5	903.1	642.95	642.95	2241	2011.5	2216.7	1578.15	1578.15
Cost Application (RM)	1.09	1.85	1.22	1.23	1.23	59.95	101.75	67.1	67.65	67.65	147.15	249.75	164.7	166.05	166.05
Cost Transportation (RM)	0.13	0.21	0.24	0.26	0.26	7.15	11.55	13.2	14.3	14.3	17.55	28.35	32.4	35.1	35.1
Total Cost (RM)	**17.82**	**16.69**	**17.88**	**13.18**	**13.18**	**980.1**	**917.95**	**983.4**	**724.9**	**724.9**	**2405.7**	**2253.15**	**2413.8**	**1779.3**	**1779.3**

Table 5.16 Cost of production (CPO) for treatment 3 on 2014 - 2018 for per palm, per acre, and per hectare of oil palm plantation

75% Inorganic: 25% Organic Fertilizer															
Fertilizer	**Year of trial (kg/palm)**					**1 Acre / 55 palm**					**1 Hectare/ (kg / 135 palms)**				
Years	**2014**	**2015**	**2016**	**2017**	**2018**	**2014**	**2015**	**2016**	**2017**	**2018**	**2014**	**2015**	**2016**	**2017**	**2018**
Sulphate of ammonia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rock phosphate	0.50	-	2.00	2.00	2.00	27.5	-	110	110	110	67.5	-	270	270	270
Muariate of potash	-	1.00	-	-	-	-	55	-	-	-	-	135	-	-	-
Kieserite	-	-	1.00	1.00	1.00	-	-	55	55	55	-	-	135	135	135
NPKMg	1.50	1.64	1.68	1.82	1.82	82.5	90.2	92.4	100.1	100.1	202.5	221.4	226.8	245.7	245.7
Organic Fertilizer	16.88	43.20	47.10	24.74	24.74	928.4	2376	2590.5	1360.7	1360.7	2278.8	5832	6358.5	3339.9	3339.9
Cost Material (RM)	18.56	2.55	20.10	12.42	12.42	1020.80	140.20	1105.50	683.10	683.10	2505.60	344.13	2713.50	1676.70	1676.70
Cost Application (RM)	0.54	1.15	0.90	0.75	0.75	29.7	63.25	49.5	41.25	41.25	83.7	178.25	139.5	116.25	116.25
Cost Transportation (RM)	0.06	0.13	0.16	0.17	0.17	3.3	7.15	8.8	9.35	9.35	8.1	17.55	21.6	22.95	22.95
Total Cost (RM)	**19.17**	**19.47**	**21.16**	**13.34**	**13.34**	**1054.35**	**1070.85**	**1163.8**	**733.7**	**733.7**	**2587.95**	**2628.45**	**2856.60**	**1800.90**	**1800.90**

Table 5.17 Cost of production (CPO) for treatment 4 on 2014 - 2018 for per palm, per acre, and per hectare of oil palm plantation

0% Inorganic: 100% Organic Fertilizer															
Fertilizer	**Year of trial (kg/palm)**					**1 Acre / 55 palm**					**1 Hectare/ (kg / 135 palms)**				
Years	**2014**	**2015**	**2016**	**2017**	**2018**	**2014**	**2015**	**2016**	**2017**	**2018**	**2014**	**2015**	**2016**	**2017**	**2018**
Sulphate of ammonia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rock phosphate	0.50	-	2.00	2.00	2.00	27.5	-	110	110	110	67.5	-	270	270	270
Muariate of potash	-	1.00	-	-	-	-	55	-	-	-	-	135	-	-	-
Kieserite	-	-	1.00	1.00	1.00	-	-	55	55	55	-	-	135	135	135
NPKMg	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Organic Fertilizer	22.50	57.60	23.75	34.11	13.74	1237.5	3168	1306.25	1876.05	755.7	3037.5	7776	3206.25	4604.85	1854.9
Cost Material (RM)	8.18	21.46	23.75	13.74	13.74	449.9	1180.3	1306.25	755.7	755.7	1104.3	2897.1	3206.25	1854.9	1854.9
Cost Application (RM)	0.23	1.00	1.90	1.50	1.50	12.65	55	104.5	82.5	82.5	31.05	135	256.5	202.5	202.5
Cost Transportation (RM)	0.56	1.47	1.64	0.64	0.64	30.8	80.85	90.2	35.2	35.2	75.6	198.45	221.4	86.4	86.4
Total Cost (RM)	**8.97**	**23.93**	**27.29**	**15.88**	**15.88**	**493.35**	**1316.15**	**1500.95**	**873.4**	**873.4**	**1210.95**	**3230.55**	**3684.15**	**2143.8**	**2143.8**

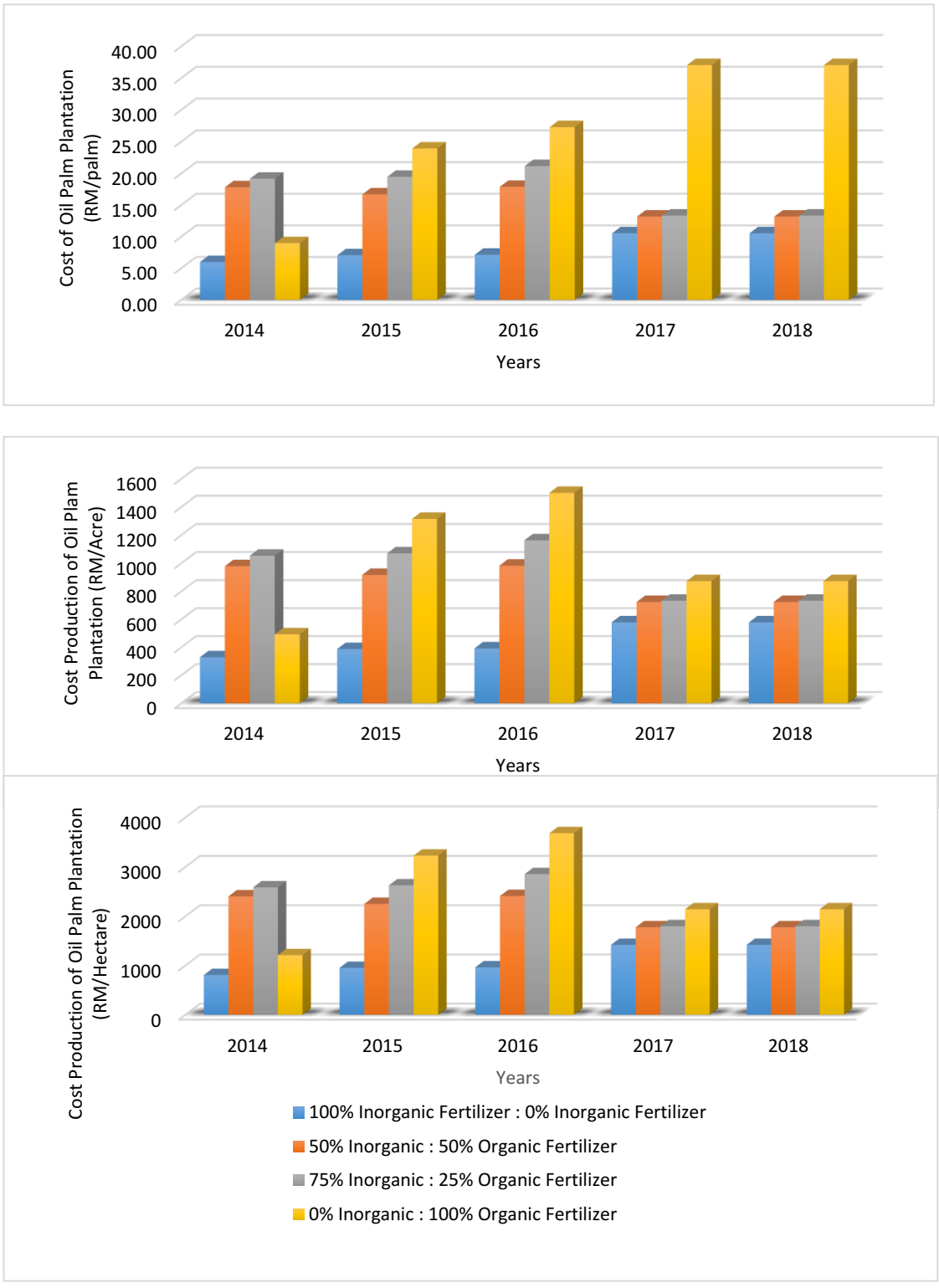


Figure 5.26 Cost of production (CPO) for treatment 1, treatment 2, treatment 3 and treatment 4 on 2014-2018 for per palm, per acre and per hectare of oil palm plantation

5.4 Conclusion

The important result from this experiment is, what happed during and after application of inorganic and inorganic fertilizer in oil palm plantation within five years. Results on soil physiochemical plant characteristic analysis showed no significantly difference with all treatments even with 100% inorganic fertilizer or 100% organic fertilizer except for potassium (K) of leaf between treatment. Result for oil extraction is a very important indicator in the assessment of application fertilizer. Proven data on oil extraction by Treatment 2 (50% inorganic fertilizer: 50% organic fertilizer), Treatment 3 (25% inorganic fertilizer: 75% organic fertilizer) and Treatment 4 (100% organic fertilizer) not effect on oil extraction of oil palm plantation compare with Treatment 1 (100% inorganic fertilizer) as commercially practiced. On microbial diversity, application of inorganic fertilizer improves microbial community in the soil. After five years application mixed inorganic fertilizer at oil palm plantation, Crenarchaeote under kingdom Archaea appear in soil at Treatment 2, 3, and 4 but not in Treatment 1 (100% inorganic fertilizer). Application of inorganic fertilizer on oil palm plantation within four years increased phyla *Bacteroidetes* and *Firmucates* as beneficial bacterial in soil.

CHAPTER 6

APPLICATION OF COMPOST IN MIXED MEDIA IMPROVED OIL PALM NURSERY'S SECONDARY ROOT STRUCTURE THEREBY REDUCING THE FERTILIZER REQUIREMENT FOR GROWTH

6.1 Introduction

Oil palm (*Elaeis guineesis*) is an important commercial agriculture crop which provides income and employment in the agricultural sector in the tropics. The waste and resources from the non-oil streams in this industry are rich in nutrients which can generate additional economic value. The empty fruit bunch (EFB) and palm oil mill effluent (POME) anaerobic sludge are two raw materials that can be converted to organic fertilizer, which can improve soil fertility and conditions the soil in oil palm plantations. The prolonged cultivation of oil palm depleted the essential nutrients and soil cation exchange capacity (CEC). This can be alleviated by the application of organic fertilizer from EFB and POME anaerobic sludge (Ashraf et al., 2017), which is an excellent nutrient-recycling strategy within the industry. The palm oil industry conventionally used topsoil in the media for the oil palm main nursery. Due to the limitation of good topsoil, application of inorganic fertilizers has been widely used which contributed to soil acidification, declined pH and low fertility of soil as media for oil palm nursery (Rosenani *et al.* 2016). An alternative growth medium is desirable to produce high quality seedlings, particularly by enhancing plant growth and root structure. It has been proposed that EFB and POME anaerobic sludge can improve soil fertility by increasing the water holding capacity, total nitrogen content, available phosphorus content, CEC and microbial activity (Ramli *et al.* 2016). In a previous

study by Siregar *et al.* (2011), the growth in the main nursery with mixed media comprising of top soil and EFB compost was similar to topsoil with the normal fertilizer application. Rosenani *et al.* (2016) reported that the use of EFB in seedling planting nursery improved soil chemical properties and vegetative growth. In addition, Albregts and Chandler (1993) reported that the level and form of the fertilizer applied affected the root and shoot morphology. The aim of this study is to determine the effect of compost produced from EFB and POME anaerobic sludge as a component in the mixed media on the growth and inorganic fertilizer requirement of oil palm main nursery plants.

6.2 Materials and methods

6.2.1 Treatments

The study was conducted in Ladang 10, Universiti Putra Malaysia with daily temperature variation of 24 – 33°C. Pressed-shredded EFB and POME anaerobic sludge obtained from Seri Hulu Langat Palm Oil Mill and FELDA Trolak Palm Oil Mill, respectively, were composted at the Biorefinery Technology Laboratory, Universiti Putra Malaysia as reported by (Baharuddin *et al.* 2009). Oil palm seeds, Dura x Psifera, were obtained from FELDA Agricultural Services Sdn. Bhd, Pusat Penyelidikan Tun Razak, Bandar Jengka, Pahang, Malaysia. Five polybag media were prepared with 100% Serdang series topsoil and compost (50% topsoil: 50% compost v/v) with different percentages of inorganic fertilizer, as shown in Table 1. The experiment was carried out in a randomized complete block design (RCBD) with four replications. The planting distance between each polybag in the block was 3 ft. x 3 ft. (triangle). One oil palm seed was sown in each polybag of 38 cm x 45 cm with equal volume per volume of media. The seedlings were watered twice a day by using the drip system and weeded manually. An inorganic fertilizer, NPK Yellow (15:15:6) was applied from week 1 (10 g/plant), week 2 (15 g/plant), weeks 3-5 (20 g/plant) and weeks 6-8 (30 g/plant), as used in conventional agricultural practice at the oil palm main nursery. Composite sample was taken from 10 seedlings per replication and 4 replications for every sampling. Table 6.1 showed treatment on this experiment, i.e. 100 % soil with 100 % inorganic fertilizer (Treatment 1) as control, 50 % soil: 50 % compost with 100 % inorganic fertilizer (Treatment 2), 50 % soil: 50 % compost with 100 % inorganic fertilizer (Treatment 3), 50 % soil: 50 % compost with 50 % inorganic

fertilizer (Treatment 4) and 50 % soil: 50 % compost with 25 % inorganic fertilizer (Treatment 5).

6.2.2 Compost production

The compost was produced as described by (Baharuddin *et al.* 2009) with a mixed ratio of 1:1 pressed-shredded empty fruit bunch (EFB) and thickened palm oil mill effluent anaerobic sludge, for a period of up to 40 days, to ensure its maturity.

6.2.3 Plant characteristic analysis

6.2.3.1 Plant growth

The growth of the seedlings was monitored at three, six and eight months after transplanting by recording the girth size, plant height, frond number, frond length and dry weight. The greenness of the oil palm seedling leaves or the chlorophyll content was measured by using a SPAD 502 Chlorophyll meter. The planting media were sent to the laboratory to be analyzed for their nutrient content at the beginning and at the end of the experiment. Leaf samples were harvested from frond number 3 for nutrient analysis at the end of the study by drying the sampled leaves. For biomass determination, the fronds and the girths were separated and dried at 70^0C in an oven until constant weight.

6.2.3.2 Soil properties and nutrient content

Soil having weight of 20.00 g was placed into a plastic bottle. Distilled water was added into the bottle. The soil was shaken intermittently for one hour and left to stand overnight. The pH was calibrated by using buffers of pH 4.00 and pH 7.00. Basic exchangeable cations were extracted and determined by electrolyte solution in 0.01 M

KCl. CEC was determined with ammonium acetate and the determination of ammonium ions in the soil was done using the colorimetric method. The total organic C content of the soil was determined by using the Walkley and Black titration method (Gelman et al., 2012). The total N content of the soil was determined using alkaline phenol and hypochlorite. In order to measure the P content, the soil sample was first digested for 1 ¾ hours by using a Block Digester at 200 ºC and the analysis of P was then done using the Auto-Analyser. Determination of soil exchangeable cations (K, Ca, Mg, and Na) was done by using 1M ammonium acetate. The potassium, magnesium and calcium determinations were done on the AAS by pipetting 2 ml of the original solution and adding 20 ml of 825 ppm Strontium nitrate by using the Auto-Diluter 111. The sodium determination was done using the original solution and reading on the AAS (Sime Darby Research, 2018).

6.2.3.3 Root analysis

The remaining soil adhering to the roots was removed by washing with water. The roots were separated according to their classes, namely the primary and the secondary roots. A digital calliper was used to measure the root diameter. The dry weights of the root samples were recorded after drying them in the oven at 70-80^0 C until constant weight (Rosenani *et al.* 2016).

6.2.4 Microbial analysis

DNA extraction from water was done by DNA extraction was carried out using the POWERSOIL^{TM} Sterivex^{TM}DNA Isolation Kit following manufacturer`s instrument (Mo Bio Laboratories, Carlsbad, CA, USA).

6.2.4.1 High throughput 16S rRNA sequencing

The high throughput 16S rRNA sequencing as described in Section 3.3.

6.2.4.2 Data analysis for bacterial community composition

High-throughput MiSeq data were processed and analysed using QIIME2. The raw paired-end reads were assembled using putty and WinSCP tool, followed by a trimming process to remove low quality and ambiguous reads. The high-quality reads were clustered into operational taxonomic units (OTUs) with 97% sequence similarity using the de novo OTU picking pipeline. The rarefied OTU tables were used as the basis for the alpha diversity measurement, and rarefaction curves were computed using the Shannon diversity metric. Beta diversity was analysed using principal coordinate analysis (PCoA) and cluster analysis using Jackknife beta-diversity and UPGMA.

6.2.4.3 Principal coordinate analysis (CPO)

Principal coordinate analysis (PCO) (Gower, 1966) was done to graphically illustrate the relationships between bacterial taxa within the phylum *Proteobacteria*, physicochemical characteristics (BOD_5 and COD) and nutrients compositions (phosphorus, ammonium, nitrogen, nitrate and potassium) of POME final discharge obtained from four different palm oil mills. Data points were clustered using correlation similarity measure to obtain a PCO scatter plot.

6.2.5 Statistical analysis

Data on the soil properties and nutrient depletion were subjected to analysis of variance (ANOVA) using the SAS Software Windows Version 8 (SAS, 2001). Tukey at $P \leq 0.05$

was used to test for significant difference between the treatments. The experiment was arranged in a Randomized Complete Block Design (RCBD) with five treatments and four replications **(Section 3.4).**

6.3 Results and discussion

6.3.1 Plant physical characteristics

Figure 6.1 shows the diagram of oil palm nursery stage comprising of the top part (leaves and rachis) and lower part (girth, primary root and secondary root). Figure 6.2 shows the plant growth after eight months under the different treatments. Table 6.2 shows the plant physical characteristic measurements taken after eight months. Table 5.2 shows plant height, girth size, chlorophyll content and frond length were significantly different on plant physical characteristic. The oil palm seedlings plant height in Treatments 2, 3, 4 and 5 planting media were significantly taller than the control seedlings in the main nursery. The biggest girth size belongs to Treatment 2 and the lowest Treatment 1. The frond lengths in Treatments 2, 3, 4 and 5 were longer and comparable with the oil palm seedlings at the main nursery in the control Treatment 1. The chlorophyll content was high in Treatment 2, followed by Treatment 3, Treatment 4, Treatment 5 and the lowest in Treatment 1. However, the frond production, and frond number of the leaflet readings taken at the end of the study did not show any significant difference in the values among the treatments tested.

Table 6.1. List of treatments used to treat oil palm seedling in this study

Treatment	Percentage soil with inorganic fertilizer
T1	100 % Soil With 100 % Inorganic fertilizer
T2	50 % Soil: 50 % Compost With 100 % Inorganic fertilizer
T3	50 % Soil: 50 % Compost With 75 % Inorganic fertilizer
T4	50 % Soil: 50 % Compost With 50 % Inorganic fertilizer
T5	50 % Soil: 50 % Compost With 25 % Inorganic fertilizer

Table 6.2 Oil palm main nursery plant characteristics under different treatments.

	Treatments	Plant height (cm)	Frond production	Girth Size (cm)	Chlorophyll content (SPAD unit)	Frond length (cm)	Frond number of leaflet (%)
T1	100 % Soil With 100 % Inorganic fertilizer	90.35±5.89b	17±0.66a	6.31±0.05b	52.49±7.75b	55.48±3.52b	24±1.25a
T2	50 % Soil: 50 % Compost With 100 % Inorganic fertilizer	101.56±7.92ab	18±0.50a	7.62±0.56b	59.66±6.33a	64.30±6.23ab	26±2.63a
T3	50 % Soil: 50 % Compost With 75 % Inorganic fertilizer	104.42±7.37a	17±0.77a	7.50±0.53b	59.02±4.93a	63.60±4.67ab	27±0.58a
T4	50 % Soil: 50 % Compost With 50 % Inorganic fertilizer	104.34±2.04a	17±0.23a	7.53±0.28b	57.89±5.73a	69.73±7.21ab	26±1.41a
T5	50 % Soil: 50 % Compost With 25 % Inorganic fertilizer	105.64±1.50a	18±0.29a	8.10±0.76a	56.05±5.16a	65.70±5.34ab	28±1.50a

Note: means with the same letter column are not significantly different at $p < 0.05$ according to Tukey (n=4)

6.3.2 Physicochemical characteristic of growth media

Table 6.3 shows the result on macronutrient analysis on media. The pH and organic carbon are significantly different between before treatment and after treatment. The pH value before treatment in 100 % soil was 4.15 and that of 50 % soil: 50 % compost was 4.5. After treatment, the pH values in Treatment 1 and Treatment 5 decreased, while treatment with mixed media 50 % soil: 50 % compost in Treatments 2, 3, and 4 showed stable values between 4.01- 4.10. Organic carbon before treatment with 50 % soil: 50 % compost had a very high value compared to 100 % soil. The CEC was not significantly different among treatments. Result on macronutrients N, total P, available P, K, Ca, Mg and Al are all significantly different at $p > 0.05$. The concentration of N in 50 % soil: 50 % compost was high compared to 100 % soil. At the end of the experiment N content in all Treatments 1, 2, 3, 4, and 5 ranged from 0.06 – 0.18 %. The same was observed in total P value in 50 % soil: 50 % compost. Total P in Treatments 2, 3, 4 and 5 are high compared to Treatment 1. Addition of compost to the media can maintain and increase the amount total of P. The result on available P has the same pattern with total P. However, a different result was observed on K, with little change before and after treatment. Results on micronutrient analysis of media, B, Zn, Mn and Si showed significant differences between before and after treatment as shown in Table 6.4. Interestingly, results on B, Fe, Zn and Mn showed treatment with 50 % soil: 50 % compost gave the highest values, in contrast with 100 % soil with the lowest values. After treatment, the results for B, Fe, Zn and Mn showed stable values in media with compost addition.

6.3.3 Dry weights of leaf, rachis, girth and root

The results presented in Table 6.5 showed that the dry weight of rachis was not significantly different among the treatments. The highest dry weight of leaves within all media mixed and the lowest on control know as Treatment 1, 100 % soil with 100 % inorganic fertilizer. Result on dry weight of girth size, total of shoot and total of root same trend with dry weight of leaves, with media mixed got high dry weight. The total root showed that Treatment 5 had the best root followed by Treatment 4, Treatment 3 and Treatment 2, with the lowest in Treatment 1 (control). Figure 6.3 shows that the primary and secondary roots were significantly different in the main nursery of the oil palm. The primary and second roots of seedlings treated with 50% compost showed better results compared to the control.

Table 6.3 Effects of compost as mixed media on macronutrient chemical characteristics of the oil palm nursery

	Treatments	pH	Org C (%)	CEC (cmol(+)/kg)	N (%)	Total P	Avail P	K Exch cmol(+)/kg	Ca	Mg	Al
	Before										
	100 % Soil	4.15±013b	1.31±0.10c	4.23±0.21a	0.15±0.15b	44.33±5.13e	10.50±1.91c	0.07±0.01a	1.30±0.12bc	0.17±0.01e	0.37±0.03b
	50 % Soil: 50 % Compost	4.5±0.08a	7.59±0.12a	4.12±0.12a	0.38±0.01a	973.33±19.76a	27.50±2.08c	0.03±0.01b	2.13±0.02a	2.11±0.03a	0.65±0.01a
	After										
T1	100 % Soil With 100 % Inorganic fertilizer	3.77±0.13c	1.20±0.13c	5.23±0.33a	0.06±0.01a	200.33±12.0d	64.00±17.32b	0.08±0.01a	1.66±0.26b	0.44±0.09de	0.20±0.02c
T2	50 % Soil: 50 % Compost With 100 % Inorganic fertilizer	4.10±0.09b	4.23±1.12b	5.10±2.36a	0.18±0.05a	466.67±17.67bc	147.00±7.73a	0.06±0.03ab	1.27±0.17c	1.37±0.02b	0.35±0.02b
T3	50 % Soil: 50 % Compost With 75 % Inorganic fertilizer	4.06±0.06b	5.12±0.85b	4.04±0.24a	0.18±0.03a	544.67±34.39b	151.75±2.06a	0.05±0.01ab	0.76±0.16d	1.00±0.36bc	0.31±0.06bc
T4	50 % Soil: 50 % Compost With 50 % Inorganic fertilizer	4.01±0.08b	4.16±1.28b	4.62±1.38a	0.15±0.03a	544.67±34.12b	166.75±18.84a	0.05±0.02ab	0.42±0.08d	0.74±0.26cd	0.27±0.11bc
T5	50 % Soil: 50 % Compost With 25 % Inorganic fertilizer	3.98±0.02bc	3.78±0.71b	4.68±1.43a	0.14±0.02a	396.33±9.01c	59.75±21.75b	0.06±0.01ab	0.66±0.10d	0.62±0.13cd	0.22±0.04c

Note: means with the same letter column are not significantly different at $p < 0.05$ according to Tukey (n=4)

Table 6.4 Effects of compost as mix media on micronutrient chemical characteristics of the oil palm nursery

	Treatments	B	Fe	Zn	Mn	Si
		mg/kg				
	Before					
	100 % Soil	0.59±0.05c	338.15c	2.37±0.34b	1.22±0.07c	125.55±10.31a
	50 % Soil: 50 % Compost	3.25±0.04a	455.46bc	27.85±7.05a	28.04±0.90c	0.23±0.02b
	After					
T1	100 % Soil With 100 % Inorganic fertilizer	0.52±0.10c	581.35ab	1.68±0.06b	157.92±6.82a	3.91±0.22b
T2	50 % Soil: 50 % Compost With 100 % Inorganic fertilizer	1.55±0.32b	517.95ab	1.98±0.07b	110.49±18.97b	5.68±0.76b
T3	50 % Soil: 50 % Compost With 75 % Inorganic fertilizer	1.62±0.23b	577.95ab	2.00±0.06b	112.43±10.43b	4.42±0.76b
T4	50 % Soil: 50 % Compost With 50 % Inorganic fertilizer	1.35±0.22b	589.56ab	1.91±0.05b	127.56±23.63ab	4.07±1.47b
T5	50 % Soil: 50 % Compost With 25 % Inorganic fertilizer	1.5±0.15b	674.77a	1.93±0.04b	128.95±16.28ab	4.44±0.61b

Note: means with the same letter column are not significantly different at $p < 0.05$ according to Tukey (n=4)

Table 6.5 Effects of compost as mix media on dry weights of seedlings in the oil palm nursery

	Treatments	Dry Weight of Leaves (g/plant)	Dry Weight of Rachis	Dry Weight of Girth (g/plant)	Total of Shoot (g/plant)	Total of Root (g/plant)	Root: Shoot
T1	100 % Soil With 100 % Inorganic fertilizer	177.27±4.50b	133.43±22.64a	102.98±12.71b	310.70±24.16c	128.93±1.59b	0.41±0.03ab
T2	50 % Soil: 50 % Compost With 100 % Inorganic fertilizer	215.00±21.32a	163.34±12.71a	118.58±25.00ab	378.37±8.66a	136.56±4.86b	0.36±0.02b
T3	50 % Soil: 50 % Compost With 75 % Inorganic fertilizer	187.13±3.91ab	132.70±16.684a	117.46±8.60ab	319.83±16.00bc	134.57±5.95b	0.42±0.04ab
T4	50 % Soil: 50 % Compost With 50 % Inorganic fertilizer	182.13±15.80b	129.30±1.78a	135.68±18.40ab	311.43±14.95c	140.83±10.74b	0.46±0.05ab
T5	50 % Soil: 50 % Compost With 25 % Inorganic fertilizer	209.73±2.29b	156.23±15.96a	152.24±19.44a	365.97±17.90b	177.07±11.95a	0.48±0.03a

Note: means with the same letter column are not significantly different at $p < 0.05$ according to Tukey (n=4)

6.3.4 Foliar nutrient

The results presented in Table 6.6 showed that the foliar nutrients from the main nursery were only significantly different for N and P. The K, Ca, Mg, S, B, Cu, Fe and Zn contents did not show any significant difference among all the planting media tested. It was postulated that the higher total N uptake in the leaf could be due to the supplementary nutrient content of the compost itself and the optimum C:N ratio (Robbins, 2013). The value for N was the highest in Treatment 2, followed by Treatments 3 and 4.

6.3.5 Cost of material

Table 6.7 shows the material cost for oil palm planting media at nursery stage. The highest cost was for 100 % soil with 100 % inorganic fertilizer (Treatment1) as control at RM6.04 per polybag, followed by 50 % soil: 50 compost with 100 % inorganic fertilizer at RM5.20 (Treatment 2). Treatment 3 with 50% soil: 50% compost with 75% inorganic fertilizer was RM4.64 per polybag while in Treatment 4 it was RM4.23. The lowest cost was in Treatment 5 at RM3.92 per polybag.

6.3.6 General characteristic of the microbial communities

Table 6.8 show, Correlation Bacteria Community composition Used MiSeq gene amplicon from 8 samples produced Compost (C15) bacteria a 5812 sequences, 100% soil with 100% inorganic fertilizer (N1) 22 archaea sequences and 60 769 sequences, 50 % soil : 50 % compost with 100% (N2) contain 12 672 sequences, 50 % soil : 50 % compost with 75% inorganic fertilizer (N3) contain 12 672 sequences, 50 % soil : 50 % compost with 50 % inorganic fertilizer (N4) contain 11 374 sequences, 50 % soil : 50 %

compost with 25 % inorganic fertilizer (N5) contain 9,882 sequences, 100 % soil before treatment contain 6 249 sequences and mixed media before treatment 8,509 sequences observed OUT. Fastq quality check tool in QIME2 pipeline revealed that these libraries represented the majority of 16S rRNA sequences present in each sample, with values ranging from 90% to 99%. Archaea were relatively rare in all soils (0.01-6.7%) but were most abundant in the hot desert sites and one of the tropical rainforests. (Cross-biome metagenomic analyses of soil microbial communities and their functional attributes). Result on oil palm nursery analysis on microbe indicate the potential microbial communities under cultivation systems (Figure 6.1 and 6.2). The major phyla observed in all samples belong to *Proteobacteria*, *Actinobacteria*, *Chloroflexi*, *Acidobacteria, Firmicutes, TM7, Planctomycetes, Verrucomicrobio and Bacteriodetes. Proteobacteria*, encompass an enormous level of morphological, physiological and metabolic diversity, and are of great importance to global carbon, nitrogen and sulfur cycling (Kersters *et al.* 2006). *Actinobacteria*, as helper bacteria on recycling and soil fertility through the involvement of many components to serve as nutrient enhancer. Phylum Chloroflexi could also play an important role in the biogeochemical chlorine cycle (Krzmarzick *et al.* 2011). Chloroflexi was found to be ecologically significant in the membrane bioreactors treating municipal wastewater and was responsible for the degradation of soluble microbial products (SMP), including organic carbohydrates and cellular materials (Miura *et al.,* 2007). *Acidobacteria,* are oligotrophic organisms abundant in carbon poor soils (Nemergut *et al.* 2010). *Firmicutes,* containing many anaerobic bacteria significantly decreases when the soils were recovered under aerobic condition. *TM7 known as Saccharibacteria*, two particular environments have generated significant interest: wastewater treatment plants and human associated TM7 (Mielczarek *et al.* 2012).

Bacteriadetes important contributors to nutrient turnover and nitrogen cycling in soil (Yousuf *et al.* 2012). *Proteobacteria, Planctomycetes, Verrucomicrobia* and *Nitrospirae. Acidobacteria* is of the most common bacterial phyla found in terrestrial ecosystems function as carbon cycle because it can degrade complex plant derived polysaccharides such as cellulose and lignin (Barns *et al.* 1999). Taxonomically, the major phyla observed in all samples belong to *Acidobacteria, Actinobacteria*, *Bacteriodetes, Chloroflexi*, *Firmicutes, Proteobacteria, Planctomycetes, Verrucomicrobia* and *Nitrospirae. Acidobacteria* is of the most common bacterial phyla found in terrestrial ecosystems function as carbon cycle because it can degrade complex plant derived polysaccharides such as cellulose and lignin (Barns *et al.* 1999). *Bacteriadetes* important contributors to nutrient turnover and nitrogen cycling in soil (Yousuf *et al.* 2012). Actinobacteria as helper bacteria shown high at Figure 6.3. Besides this, several other endophytic actinobacteria exhibited N-fixing ability which includes *Arthrobacter, Agromyces, Corynebacterium, Mycobacterium, Micromonospora, Propionibacteria, and Streptomyces* (Sellstedt *et al.* 2013). Figure 6.3 show Streptomyces higher in all treatments. This nutrient cycling capacity makes them as an ideal candidate for natural fertilizers (Jog *et al.* 2016). In addition, the metal mobilizing ability can be applied for biofortification approaches for enhancing seed mineral nutrients such as Fe, Zn, and Si. However, limited studies are available on legumes. A recent study had revealed that arbuscular mycorrhizal fungal colonization on chickpea roots enhanced the crop growth, and grain Fe and Zn contents (Pellegrino *et al.* 2014). Although bacterial copy numbers generally did not correlate well with soil chemical and physical properties, there were strong correlations between several bacterial populations and soil properties. Populations of *Acidobacteria* were negatively correlated with pH. Furthermore, *Acidobacteria* and *Planctomycetes* tended

to be positively correlated with various pools of soil and microbial biomass C and N while *Bacteroidetes* which have a positive relationship with C mineralization. As the name implies, *Acidobacteria* tend to be acidophilic and are often found to be strongly negatively correlated with soil pH Figure 6.4. It is not surprising, then, that we found *Acidobacteria* to be negatively correlated with pH here as well. It has been argued that *Acidobacteria* tend to be oligotrophic and that *Bacteroidetes* tend to be copiotrophic, meaning that populations of *Acidobacteria* have an inverse relationship with C mineralization in contrast. Both *Acidobacteria* and *Bacteroidetes* are very broad phyla so it is possible that our soil selected for populations different than those in the samples examined by (Fierer *et al.* 2006). Soils close to neutral had the highest diversity levels, whereas soils that were either very basic or acidic had lower levels of diversity. However, it is possible that the low diversity of the cold desert soils is not directly related to their very high pH levels, but rather due to their high salinities, negligible plant carbon inputs, or the extreme moisture and temperature conditions encountered at those sites. More generally, the results shown here confirm the broad scale patterns we would expect based on pH differences; high pH soils typically have higher relative abundances of *Actinobacteria* and *Bacteroidetes* with lower abundances of *Acidobacteria*. Acidic soil has a high abundance of *Acidobacteria* but these *Acidobacteria* belong to the class *Chloracidobacteria* that is distinct from the *Acidobacteria* group (*Solibacteres*), which dominates in low pH soils; this is a pattern we would expect based on the results reported in Jones et al. Factors other than pH may also be driving the bacterial community patterns evident in taxa known to be tolerant of low moisture conditions, including *Actinobacteria*.

6.4 Conclusion

Mixed media with 50 % soil: 50 % compost can be used as media and slow release fertilizer for oil palm nursery.

Table 6.6 Effects of compost as mix media on foliar nutrient in the oil palm nursery.

	Treatments	Total N (%)	P	K	Ca	Mg	Cl	S	B	Cu	Fe	Zn
					Exch cmol(+)/kg				mg/kg			
T1	100 % Soil With 100 % Inorganic fertilizer	2.71±0.12ab	0.17±0.01ab	0.90±0.03a	0.63±0.07a	0.26±0.03a	0.88±0.04a	0.13±0.01b	16.00±0.82a	1.55±0.36a	96.11±3.15a	19.32±0.84a
T2	50 % Soil: 50 % Compost With 100 % Inorganic fertilizer	2.88±0.06a	0.18±0.01a	1.00±0.08a	0.65±0.09a	0.28±0.02a	0.88±0.03a	0.14±0.01a	15.75±1.26a	1.74±0.55a	97.81±7.80a	20.11±1.18a
T3	50 % Soil: 50 % Compost With 75 % Inorganic fertilizer	2.84±0.12ab	0.17±0.01ab	0.91±0.02a	0.73±0.05a	0.25±0.01a	0.88±0.02a	0.14±0.01a	16.00±0.82a	2.38±0.27a	101.93±3.76a	21.03±1.05a
T4	50 % Soil: 50 % Compost With 50 % Inorganic fertilizer	2.84±0.03ab	0.17±0.01ab	0.91±0.06a	0.72±0.04a	0.26±0.01a	0.88±0.01a	0.15±0.01a	15.50±1.00a	2.34±0.32a	101.55±3.44a	21.26±0.38a
T5	50 % Soil: 50 % Compost With 25 % Inorganic fertilizer	2.67±0.03b	0.16±0.01b	0.91±0.04a	0.66±0.11a	0.24±0.01a	0.90±0.01a	0.14±0.01a	14.5±1.00a	2.22±0.41a	98.91±2.60a	19.21±1.41a

Note: means with the same letter column are not significantly different at $p < 0.05$ according to Turkey (n = 4)

Table 6.7 Material cost per polybag of planting media

Treatment	Percentage soil with inorganic fertilizer	RM / Polybag
T1	100% Soil With 100% Inorganic fertilizer	RM6.04
T2	50% Soil: 50% Compost With 100% Inorganic fertilizer	RM5.20
T3	50% Soil: 50% Compost With 75% Inorganic fertilizer	RM4.64
T4	50% Soil: 50% Compost With 50% Inorganic fertilizer	RM4.23
T5	50% Soil: 50% Compost With 25% Inorganic fertilizer	RM3.92

Topsoil =RM160/ton, Compost =RM350/ton, NPK Blue = RM1300/ton and NPK Yellow = RM1200/ton.

Table 6.8. Summary of description treatment from each respective on kingdom Archaea and Bacteria

Sample	Description	k__Archaea	k__Bacteria
C15	Organic Fertilizer / Media	0	5812
T1	100 % Soil: 100 % Inorganic Fertilizer	22	45741
T2	50 % soil: 50 % Compost – 100 % Inorganic Fertilizer	0	60769
T3	50 % soil: 50 % Compost – 75 % Inorganic Fertilizer	0	12672
T4	50 % soil: 50 % Compost – 50 % Inorganic Fertilizer	0	11374
T5	50 % soil: 50 % Compost – 25 % Inorganic Fertilizer	0	9882
S100	100 % Soil - Before treatment	0	6249
S50	50 % Soil: 50 % Compost Before treatment	0	8509

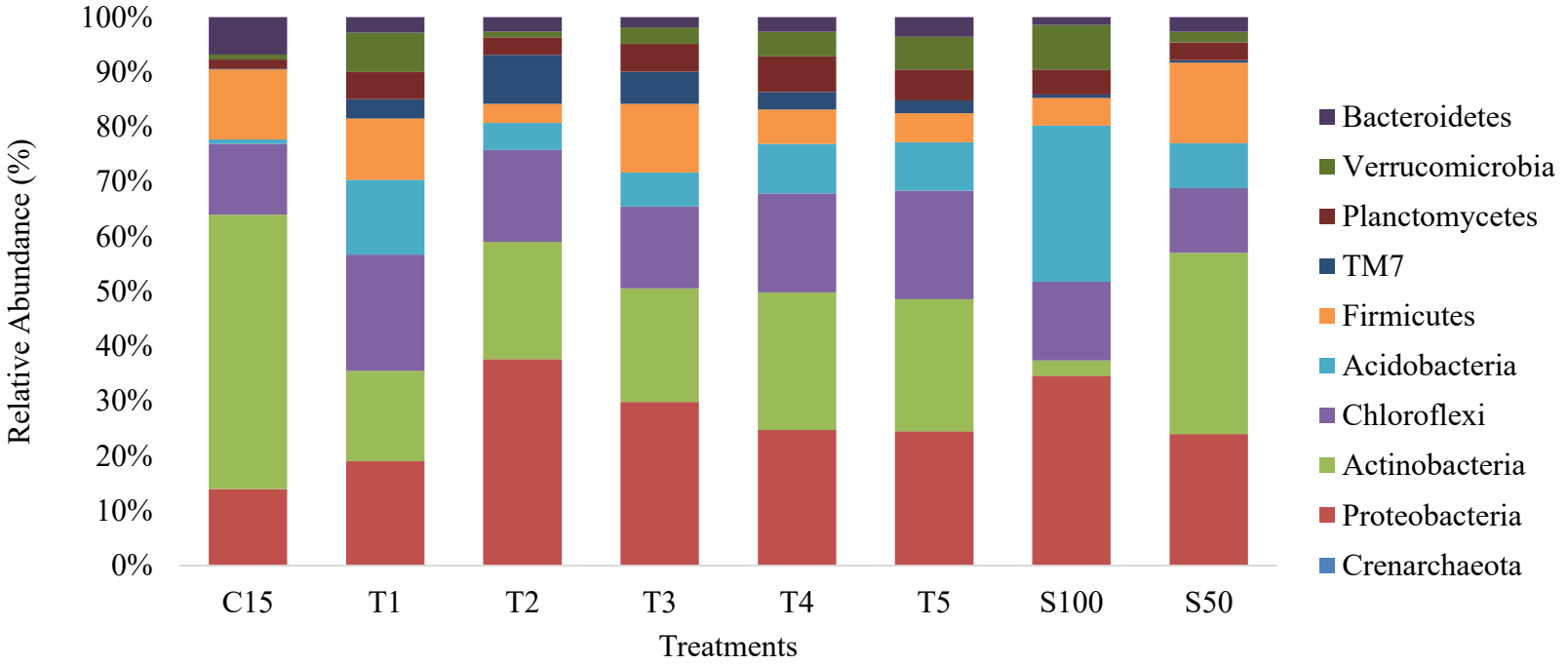


Figure 6.1 Relative abundance of dominant bacteria taxa in oil palm

C15- compost
T1 -100 % soil: 100 % compost with 100 % inorganic fertilizer
T2 - 50 % soil: 50 % compost with 100 % inorganic fertilizer
T3 - 50 % soil: 50 % compost with 75 % inorganic fertilizer
T4 – 50 % soil: 50 % compost with 50 % inorganic fertilizer
T5 – 50 % soil: 50 % compost with 25 % inorganic fertilizer
S100 – 100 % soil S50 – 50 % soil: 50 % compost

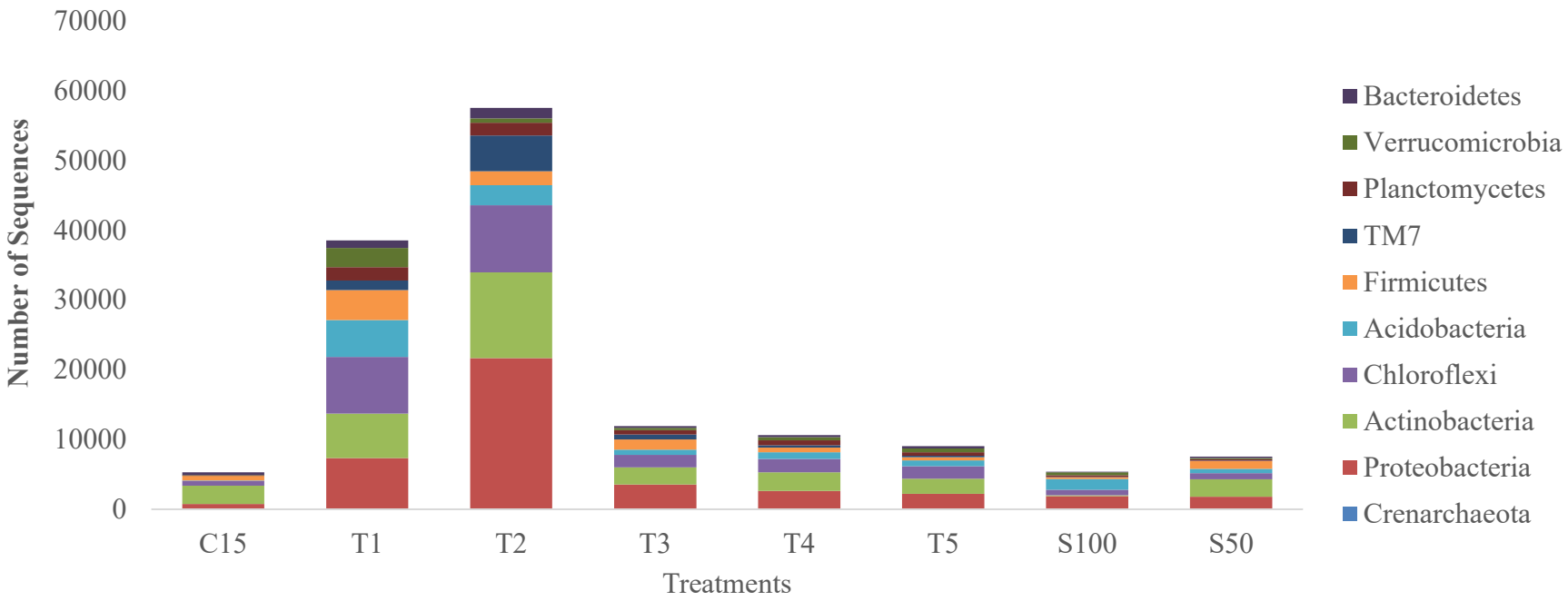


Figure 6.2 Relative abundance of dominant bacteria taxa in oil palm

C15- compost
T1 -100 % soil: 100 % compost with 100 % inorganic fertilizer
T2 – 50 % soil: 50 % compost with 100 % inorganic fertilizer
T3 -50 % soil: 50 % compost with 75 % inorganic fertilizer
T4 – 50 % soil: 50 % compost with 50 % inorganic fertilizer
T5 – 50 % soil: 50 % compost with 25 % inorganic fertilizer
S100 – 100 % soil S50 – 50 % soil: 50 % compost

Figure 6.3 Heatmap on phylum *Actinobacteria*

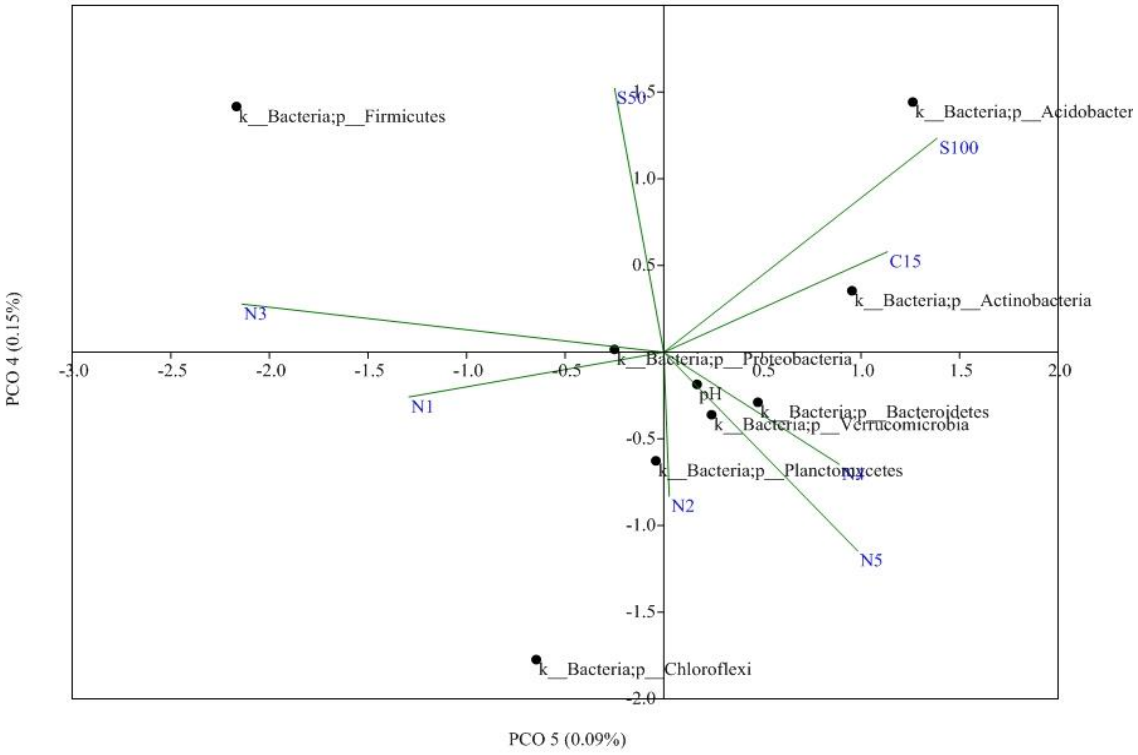


Figure 6.4 Principal Coordinate Analysis (PCO) of ecological distance between microbe on phyla and pH

CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

7.1 Conclusions

In this research compost produced from empty fruit bunch (EFB) and POME anaerobic sludge as organic fertilizer has been proposed as an partial substitute fertilizer to inorganic fertilizer for industrial use. Application of organic fertilizer compared to inorganic fertilizer is reported here, which is comparable in terms of cost and improved the performance of oil palm nursery and oil palm plantation. Overall, it can be concluded that;

1. Based on the result on nitrogen in soil after 25 years application of inorganic fertilizer in oil palm plantation, the value is very low from standard soil in oil palm. Low cation exchange capacity, and high aluminium, silica, zinc and ferum indicated that the soil was in stressed condition. Aluminium, ferum and zinc are important indicators that the soil is in stressed condition. Two important findings on soil microbial diversity after 25 years application inorganic fertilizer are decreased *Firmicutes,* which is an important soil borne diseases suppression, and decreased *Bacteriodete*s as an indicator of soil health normally associated with forest soil.

2. Application of 100% inorganic fertilizer, 50% inorganic with 50% inorganic fertilizer, 25% inorganic fertilizer with 75% organic fertilizer and 0% inorganic fertilizer with 100% organic fertilizer are comparable with mixed inorganic fertilizer on plant characteristic, soil, foliar, oil yield and microbial diversity.

The soil physiochemical characteristics were not significantly different in all treatments compared to 100% inorganic fertilizer. It was the same on foliar characteristics, which was only significantly different on P, K S, Ci, Fe and Zn. The physical characteristics were not significantly different after 5 years planting oil palm plantation means that inorganic fertilizer application did not effect the plant growth. Result on oil yield showed that even for 100% organic fertilizer, the oil yield is as good as with100% inorganic fertilizer. On the soil microbial diversity, the best microbial diversity and composition was in 50% inorganic fertilizer with 50 % organic fertilizer. Regarding the economic statistical analysis, 50% inorganic fertilizer with 50% organic fertilizer is comparable in cost with 100% inorganic fertilizer.

3. Mixed media with 50% soil: 50% compost can be used as media and slow release fertilizer for oil palm nursery. Media for plant is important in agro-ecosystems, such as organic material turnover, nutrient cycling and microbial activity. Microbes could promote crop growth and plant hormones and increase nutrient availabilityand, as a consequence, benefit plant quality and productivity.

7.2 Recommendations for future research

1. Further investigations on the application of 50% inorganic fertilizer with 50% organic fertilizer as pellet could be carried out for ease of application in the field.

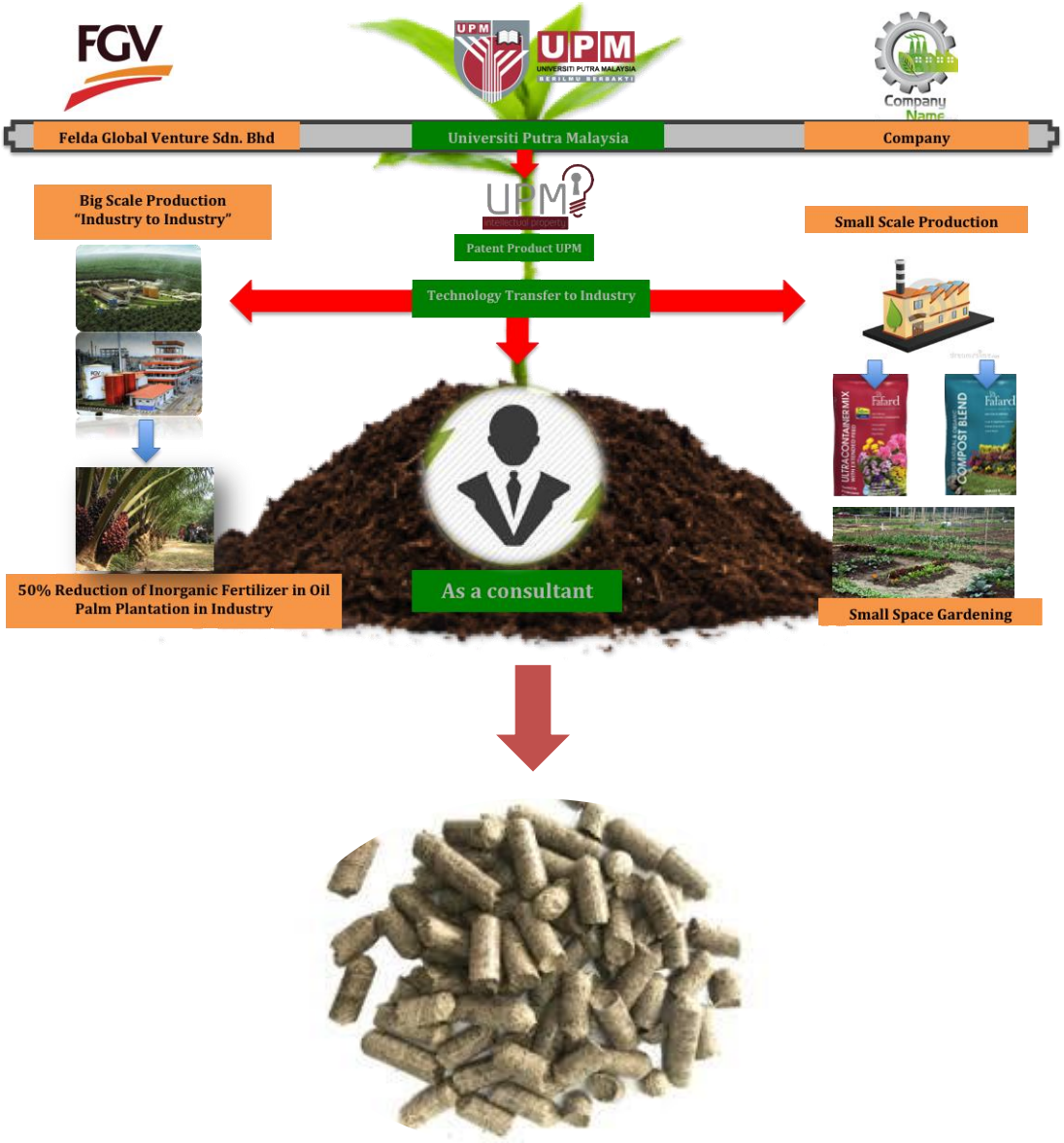


Figure 7.1 Strategy application 50% inorganic fertilizer: 50% organic fertilizer in pellets form

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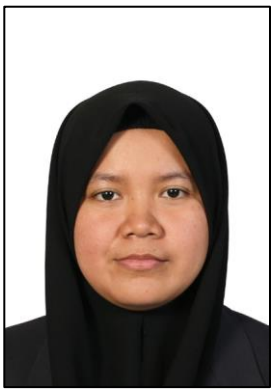
BIODATA OF STUDENT



Siti Suliza binti Salamat, born on September 21st, 1984 in Sri Medan Batu Pahat, Johor. She is daughter to Hj. Salamat bin Sanadi and Khatijah binti Damin and the six children in her eight siblings. She received her primary education in Sekolah Rendah Kebangsaan Seri Pasir Sri Medan Batu Pahat, Johor. The author was then offered to continue her secondary education at Sekolah Menengah Kebangsaan Seri Medan, Sri Medan Batu Pahat, Johor for 5 years (1997-2001) for PMR (Peperiksaan Menengah Rendah) and Sijil Pelajaran Malaysia (SPM). In 2003, she managed to further her studies up six in Sijil Tinggi Pelajaran Malaysia (STPM) for 1 years in Sekolah Menengah Kebangsaan Tongkang Pechah. In 2004 she takes second times for STPM again in private.

In 2005 further her studies in Bachelor of Horticulture, UPM for 4 year by the Faculty of Agriculture. In 2009, she started her Master degree programmed at the Institute of Tropical Agriculture, UPM with the Master of Science.

She started his Doctor of Philosophy (PhD) study in the field of Environmental Biotechnology, Faculty of Biotechnology and Biomolecular Sciences Universiti Putra Malaysia (UPM) since February, 2014. She started his Dual Degree Program (DDP) between UPM and KYUTECH, Japan since October 2014. She

received a scholarship from MyPhD from Ministry of Higher Education (MOHE) for Malaysia and Japan Student Services Organization (JASSO) & JICA at Japan. This research collaboration with Federal Land Development Authority (FELDA) and Malaysia Genome Institute (MGI). The result of his research is described in this thesis. During her candidature, she had participated on the local and international attachment programs and conferences.